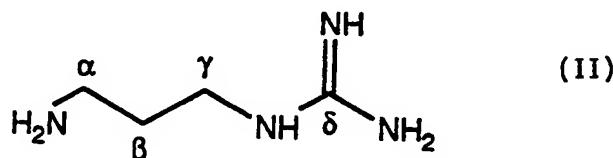
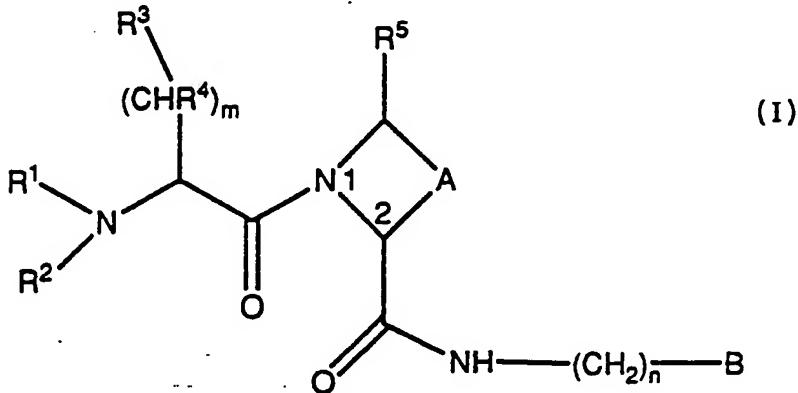




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(54) Title: NEW PEPTIDE DERIVATIVES



(57) Abstract

The invention relates to new competitive inhibitors of thrombin, their synthesis, pharmaceutical compositions containing the compounds as active ingredients, and the use of the compounds as anticoagulants for prophylaxis and treatment of thromboembolic diseases, according to formula (I) wherein A represents a methylene group, an ethylene group or a propylene group, which may be substituted or A represents $-\text{CH}_2\text{-O-CH}_2-$, $-\text{CH}_2\text{-S-CH}_2-$, $-\text{CH}_2\text{-SO-CH}_2-$, or A represents $-\text{CH}_2\text{-O-}$, $-\text{CH}_2\text{-S-}$, $-\text{CH}_2\text{-SO-}$, with the heteroatom functionality in position 4, or n is an integer 2 to 6; and B represents $-\text{N}(\text{R}^6)\text{-C}(\text{NH})\text{-NH}_2$, wherein R⁶ is H or a methyl group, or B represents $-\text{S-C}(\text{NH})\text{-NH}_2$, or $-\text{C}(\text{NH})\text{-NH}_2$. Further described is new use in synthesis of pharmaceutical compounds of a compound of formula (II).

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New peptide derivatives5 DESCRIPTION

This invention relates to new competitive inhibitors of thrombin, their synthesis, pharmaceutical compositions containing the compounds as active ingredients, and the
10 use of the compounds as anticoagulants for prophylaxis and treatment of thromboembolic diseases such as venous thrombosis, pulmonary embolism, arterial thrombosis, in particular myocardial infarction and cerebral thrombosis, general hypercoagulable states and local hypercoagulable states, e.g.
15 following angioplasty and coronary bypass operations.

The invention also relates to novel use of a compound as a starting material in synthesis of a serine protease inhibitor. Furthermore the invention relates to a novel
20 structural fragment in a serine protease inhibitor.

BACKGROUND

Blood coagulation is the key process involved in both
25 haemostasis (i.e. prevention of blood loss from a damaged vessel) and thrombosis (i.e. the pathological occlusion of a blood vessel by a blood clot). Coagulation is the result of a complex series of enzymatic reactions, where one of the final steps is conversion of the proenzyme prothrombin to the
30 active enzyme thrombin.

Thrombin plays a central role in coagulation. It activates platelets, it converts fibrinogen into fibrin monomers, which polymerise spontaneously into filaments, and it activates
35 factor XIII, which in turn crosslinks the polymer to insoluble fibrin. Thrombin further activates factor V and factor VIII in a positive feedback reaction. Inhibitors of thrombin are therefore expected to be effective

anticoagulants by inhibition of platelets, fibrin formation and fibrin stabilization. By inhibiting the positive feedback mechanism they are expected to exert inhibition early in the chain of events leading to coagulation and thrombosis.

5

PRIOR ART

Inhibitors of thrombin based on the amino acid sequence around the cleavage site for the fibrinogen A α chain were 10 first reported by Blombäck et al in J. Clin. Lab. Invest. 24, suppl 107, 59, (1969), who suggested the sequence Phe-Val-Arg (P9-P2-P1, herein referred to as the P3-P2-P1 sequence) to be the best inhibitor.

15 In US 4,346,078 (Richter Gedeon Végészeti Gyár R T, priority date 7.10.1980) and in Peptides 1983 by Walter de Gruyter & Co, Berlin, pp 643-647, S. Bajusz et al described the thrombin inhibitor H-DPhe-Pro-Agm, a dipeptidyl derivative with an aminoalkyl guanidine in the P1-position.

20 S. Bajusz et. al. also reported in J. Med. Chem. 1990, 33, 1729-1735 and in EP-A2-0,185,390 (Richter Gedeon Végészeti Gyár R T) (priority date 21.12.84) that replacing the agmatine with an arginine aldehyde gave a thrombin inhibitor 25 which had much higher potency.

The reason for the increased activity of this thrombin inhibitor is thought possibly to be due to interaction of the aldehyde function with the Ser-OH in the active site of the 30 enzyme forming a hemiacetal. It is not conceivable to have the same type of interaction in the dipptide derivative H-DPhe-Pro-Agm since it does not have an amino acid derivative with a carbonyl group in the P1-position.

35 In other work in the thrombin inhibitor field, inhibitors of serine proteases that are based on electrophilic ketones instead of aldehydes in the P1-position include the

following:

E. N. Shaw et al. (Research Corporation) US-4,318,904
(priority date 25.04.80) describing peptide chloro-methyl
5 ketones e.g. H-DPhe-Pro-Arg-CH₂Cl.

M. Szelke and D.M. Jones in EP-A1-0,118,280, (priority date
4.3.83) describing compounds derived from the P₃ - P_{2'}
pentapeptide sequence of the fibrinogen A α chain in which the
10 scissile P₁ - P_{1'} peptide bond was replaced with the
-CO-CH₂-moiety, forming a keto isostere to the corresponding
peptides.

M. Kolb et. al. (Merrell-Dow) EP-A2-0,195,212 (Priority date
15 4.2.85) describing peptidyl α -keto esters and amides.

B. Imperiali and R.H. Abeles, Biochemistry 1986. 25. 3760
describing peptidyl fluoroalkyl ketones.

20 D. Schirlin et al. (Merrell-Dow) EP-A1-0,362,002 (priority
date 1.9.88) describing fluoroalkylamide ketones.

P. Bey et al., (Merrell-Dow) EP-A2-0,364,344 (priority date
1.9.88) describing α,β,δ - triketo compounds.

25 Ueda et al., Biochem. J. 1990, 265, 539 also describing
peptidyl fluoroalkyl ketones.

Inhibitors of thrombin based on C-terminal boronic acid
30 derivatives of arginine and isothiouronium analogues thereof
have been reported by A.D Kettner et al. (Du Pont)
EP-A2-0,293,881 (priority dates 5.6.87 and 6.4.88).

An object of the present invention is to provide novel and
35 potent thrombin inhibitors with competitive inhibitory
activity towards their enzyme i.e. causing reversible
inhibition. A further object is to obtain inhibitors which

are orally bioavailable and selective in inhibiting thrombin over other serine proteases. Stability, duration of action, and low toxicity at therapeutic dosages are still further objects of the invention.

5

DISCLOSURE OF THE INVENTION

Compounds

10 Compounds of the invention relate to the peptide sequence of human fibrinogen A α chain representing modified sub-sites P₉, P₂ and P₁:

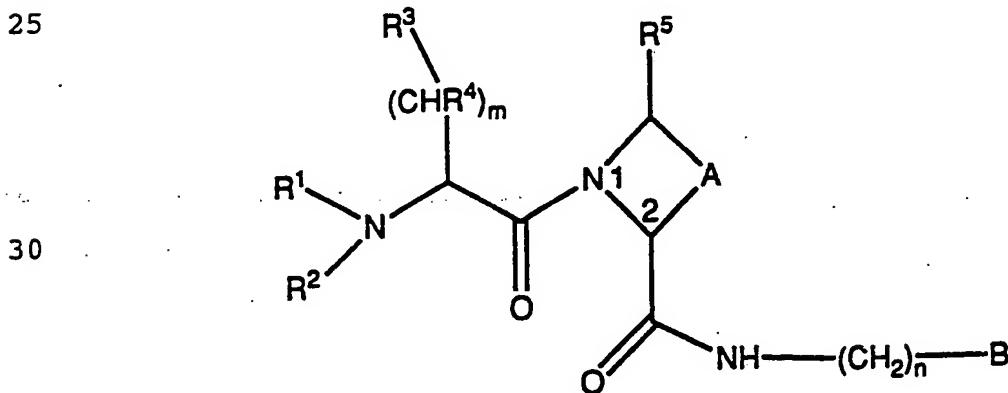
P₉
H-Ala-Asp-Ser-Gly-Glu-Gly-Asp-Phe-Leu-Ala-

15

P₂ P₁ ↓ P_{1'} P_{2'} P_{3'}
-Glu-Gly-Gly-Val-Arg—Gly-Pro-Arg-Val-

According to the invention it has been found that compounds
20 of the general Formula I, either as such or in the form of physiologically acceptable salts, and including stereoisomers, are potent inhibitors of thrombin:

25



30

35

Formula I

wherein:

A represents a methylene group, or

5

A represents an ethylene group and the resulting 5-membered ring may or may not carry one or two fluorine atoms, a hydroxy group or an oxo group in position 4, or may or may not be unsaturated, or

10

A represents $-\text{CH}_2\text{-O-}$, $-\text{CH}_2\text{-S-}$, $-\text{CH}_2\text{-SO-}$, with the heteroatom functionality in position 4, or

15

A represents a n-propylene group and the resulting 6-membered ring may or may not carry in position 5 one fluorine atom, a hydroxy group or an oxo group, carry two fluorine atoms in one of positions 4 or 5 or be unsaturated in position 4 and 5, or carry in position 4 an alkyl group with 1 to 4 carbon atoms, or

20

A represents $-\text{CH}_2\text{-O-CH}_2\text{-}$, $-\text{CH}_2\text{-S-CH}_2\text{-}$, $-\text{CH}_2\text{-SO-CH}_2\text{-}$;

25

R¹ represents H, an alkyl group having 1 to 4 carbon atoms, a hydroxyalkyl group having 2-3 carbon atoms or R¹¹OOC-alkyl-, where the alkyl group has 1 to 4 carbon atoms and R¹¹ is H or an alkyl group having 1 to 4 carbon atoms or an alkylene group having 2-3 carbon atoms intramolecularly bound alpha to the carbonyl group in R¹, or

30

R¹ represents R¹²OOC-1,4-phenyl-CH₂-, where R¹² is H or an alkyl group having 1 to 4 carbon atoms, or

35

R¹ represents R¹³-NH-CO-alkyl-, where the alkyl group has 1 to 4 carbon atoms and is possibly substituted alpha to the carbonyl with an alkyl group having 1 to 4 carbon atoms and where R¹³ is H or an alkyl group having 1 to 4 carbon atoms or -CH₂COOR¹² where R¹² is as defined above, or

R¹ represents R¹²OOC-CH₂-OOC-alkyl-, where the alkyl group has 1 to 4 carbon atoms and is possibly substituted alpha to the carbonyl with an alkyl group having 1 to 4 carbon atoms and where R¹² is as defined above, or

5

R¹ represents CH₃SO₂-, or

R¹ represents R¹²OCOCO- where R¹² is as defined above, or

10 R¹ represents -CH₂PO(OR¹⁴)₂, -CH₂SO₃H or -CH₂-(5-(1H)-tetrazolyl) where R¹⁴ is, individually at each occurrence, H, methyl or ethyl;

15 R² represents H or an alkyl group having 1 to 4 carbon atoms or R²¹OOC-alkyl-, where the alkyl group has 1 to 4 carbon atoms and is possibly substituted in the position which is alpha to the carbonyl group, and the alpha substituent is a group R²²-(CH₂)_p-, wherein p = 0-2 and R²² is methyl, phenyl, OH, COOR²¹, and R²¹ is H or an alkyl group having 1 to 4

20 carbon atoms;

m is 0, 1 or 2, R³ represents a cyclohexyl group and R⁴ represents H, or

25 m is 1 and R³ represents a cyclohexyl or phenyl group and R⁴ forms an ethylene bridge together with R¹, or

m is 1 and R³ and R⁴ each represents a cyclohexyl or phenyl group;

30

R⁵ represents H or an alkyl group having 1 to 4 carbon atoms;

n is an integer 2 to 6; and

35 B represents -N(R⁶)-C(NH)-NH₂, wherein R⁶ is H or a methyl group, or

B represents $-S-C(NH)-NH_2$, or $-C(NH)-NH_2$.

An alkyl group may be straight or branched unless specified otherwise. Alkyl groups having 1 to 4 carbon atoms are

5 methyl, ethyl, n-propyl, i-propyl, n-butyl, i-butyl, s-butyl and t-butyl. When unsaturation is referred to, a carbon-carbon double bond is intended. Abbreviations are listed at the end of this specification.

10 According to a preferred embodiment the invention relates to compounds of Formula I, wherein R^1 represents $R^{11}OOC$ -alkyl-, where the alkyl group has 1 to 4 carbon atoms and R^{11} is H. Of those compounds, the compounds where A is ethylene and R^5 is H or an alkyl group having 1 to 4 carbon atoms,

15 particularly those where R^5 is H are preferred.

Of the compound of Formula I, those compounds where R^3 is cyclohexyl, m is 1 or 2, particularly m is 1 and R^4 is H constitute another preferred subclass.

20 Another preferred group of compounds are the compounds where A is n-propylene and the resulting 6-membered ring may or may not carry in position 4 an alkyl group with 1 to 4 carbon atoms, and R^5 is H or an alkyl group having 1 to 4 carbon atoms, particularly those where R^5 is H.

According to another preferred embodiment n is 3.

Compounds of Formula I having S-konfiguration on the α -amino acid in the P2-position are preferred ones, of those compounds also having R-konfiguration on the α -amino acid in the P3-position are particularly preferred ones.

Preferred compounds of the invention are:

| <u>Example No.</u> | <u>Compound</u> |
|--------------------|--|
| 1 | H-(R)Cha-Pro-Agm |
| 2 | Me-(R)Cha-Pro-Agm |
| 5 3 | HO-(CH ₂) ₃ -(R)Cha-Pro-Agm |
| 4 | HOOC-CH ₂ -(R)Cha-Pro-Agm |
| 5 | ⁱ PrOOC-CH ₂ -(R)Cha-Pro-Agm |
| 6 | HOOC-CH ₂ -(Me)-(R)Cha-Pro-Agm |
| 7 | HOOC-(R,S)CH(Me)-(R)Cha-Pro-Agm |
| 10 8 | HOOC-(RorS)CH(Me)-(R)Cha-Pro-Agm/a |
| 9 | HOOC-(RorS)CH(Me)-(R)Cha-Pro-Agm/b |
| 10 | HOOC-(RorS)CH(ⁿ Pr)-(R)Cha-Pro-Agm/a |
| 11 | HOOC-(RorS)CH(ⁿ Pr)-(R)Cha-Pro-Agm/b |
| 12 | HOOC-(RorS)CH(Ph)-(R)Cha-Pro-Agm/b |
| 15 13 | HOOC-(R,S)CH(CH ₂ CH ₂ Ph)-(R)Cha-Pro-Agm |
| 14 | HOOC-(RorS)CH(CH ₂ CH ₂ Ph)-(R)Cha-Pro-Agm/a |
| 15 | HOOC-CH ₂ -CH ₂ -(R)Cha-Pro-Agm |
| 16 | EtOOC-CO-(R)Cha-Pro-Agm |
| 17 | (R,S)Bla-(R)Cha-Pro-Agm |
| 20 18 | HOOC-(RorS)CH(CH ₂ CH ₂ Ph)-(R)Cha-Pro-Agm/b |
| 19 | H-(R)Cha-Pro-Nag |
| 20 | ⁿ Bu-(R)Cha-Pro-Nag |
| 21 | HO-(CH ₂) ₃ -(R)Cha-Pro-Nag |
| 22 | HOOC-CH ₂ -(R)Cha-Pro-Nag |
| 25 23 | EtOOC-CH ₂ -(R)Cha-Pro-Nag |
| 24 | ⁱ PrOOC-CH ₂ -(R)Cha-Pro-Nag |
| 25 | ^t BuOOC-CH ₂ -(R)Cha-Pro-Nag |
| 26 | HOOC-CH ₂ -OOC-CH ₂ -(R)Cha-Pro-Nag |
| 27 | H ₂ N-CO-CH ₂ -(R)Cha-Pro-Nag |
| 30 28 | HOOC-CH ₂ -NH-CO-CH ₂ -(R)Cha-Pro-Nag |
| 29 | (HOOC-CH ₂) ₂ -(R)Cha-Pro-Nag |
| 30 | HOOC-CH ₂ -(Me)-(R)Cha-Pro-Nag |
| 31 | HOOC-CH ₂ -(nBu)-(R)Cha-Pro-Nag |
| 32 | HOOC-(R,S)CH(Me)-(R)Cha-Pro-Nag |
| 35 33 | HOOC-(RorS)CH(Me)-(R)Cha-Pro-Nag/a |
| 34 | HOOC-(RorS)CH(Me)-(R)Cha-Pro-Nag/b |
| 35 | EtOOC-(R,S)CH(Me)-(R)Cha-Pro-Nag |

36 HOOC-(RorS)CH(ⁿPr)-(R)Cha-Pro-Nag/a
 37 HOOC-(R)CH(CH₂-OH)-(R)Cha-Pro-Nag
 38 HOOC-(R,S)CH(Ph)-(R)Cha-Pro-Nag
 39 HOOC-(S)CH(CH₂CH₂Ph)-(R)Cha-Pro-Nag
 5 40 HOOC-(R)CH(CH₂CH₂Ph)-(R)Cha-Pro-Nag
 41 HOOC-CH₂-CH₂-(R)Cha-Pro-Nag
 42 EtOOC-CH₂-CH₂-(R)Cha-Pro-Nag
 43 HOOC-(CH₂)₃-(R)Cha-Pro-Nag
 44 EtOOC-(CH₂)₃-(R)Cha-Pro-Nag
 10 45 HOOC-CO-(R)Cha-Pro-Nag
 46 MeOOC-CO-(R)Cha-Pro-Nag
 47 (R,S)Bla-(R)Cha-Pro-Nag
 48 HOOC-(R,S)CH(CH₂COOH)-(R)Cha-Pro-Nag
 49 MeOOC-(R,S)CH(CH₂COOMe)-(R)Cha-Pro-Nag
 15 50 HOOC-Ph-4-CH₂-(R)Cha-Pro-Nag
 51 (HO)₂P(O)-CH₂-(R)Cha-Pro-Nag
 52 EtO(HO)P(O)-CH₂-(R)Cha-Pro-Nag
 53 (EtO)₂P(O)-CH₂-(R)Cha-Pro-Nag
 54 HOOC-CH₂-(R)Cha-Pro-Mag
 20 55 H-(R,S)Pro(3-Ph)-Pro-Agm
 56 H-(R,S)Pro(3-(trans)Ch)-Pro-Agm
 57 HOOC-CH₂-(R,S)Pro(3-(trans)Ph)-Pro-Agm
 58 HOOC-CH₂-(R,S)Pro(3-(trans)Ph)-Pro-Nag
 59 HOOC-CH₂-(R)Cha-Pic-Agm
 25 60 HOOC-CH₂-(Me)(R)Cha-(R,S)Pic-Agm
 61 HOOC-(R,S)CH(Me)-(R)Cha-Pic-Agm
 62 HOOC-(RorS)CH(Me)-(R)Cha-Pic-Agm/a
 63 HOOC-(RorS)CH(Me)-(R)Cha-Pic-Agm/b
 64 HOOC-CH₂-CH₂-(R)Cha-Pic-Agm
 30 65 H-(R)Cha-Pic-Nag
 66 Me-(R)Cha-(R,S)Pic-Nag
 67 HOOC-CH₂-(R)Cha-Pic-Nag
 68 MeOOC-CH₂-(R)Cha-Pic-Nag
 69 iPrOOCH₂-(R)Cha-Pic-Nag
 35 70 HOOC-CH₂-(Me)(R)Cha-(RorS)Pic-Nag/b
 71 HOOC-(R,S)CH(Me)-(R)Cha-(R,S)Pic-Nag
 72 HOOC-(RorS)CH(Me)-(R)Cha-(RorS)Pic-Nag/c

| | | |
|----|----|---|
| | 73 | HOOC-(RorS)CH(Me)-(R)Cha-(RorS)Pic-Nag/d |
| | 74 | HOOC-CH ₂ -CH ₂ -(R)Cha-Pic-Nag |
| | 75 | HOOC-CH ₂ -(R)Cha-(R,S)Mor-Agm |
| 5 | 76 | HOOC-CH ₂ -(R)Cha-(RorS)Mor-Nag |
| | 77 | H-(R)Cha-Aze-Nag |
| | 78 | HOOC-CH ₂ -(R)Cha-Aze-Nag |
| | 79 | H-(R)Cha-Pro(5-(S)Me)-Nag |
| | 80 | HOOC-CH ₂ -(R)Cha-Pro(5-(S)Me)-Nag |
| 10 | 81 | HOOC-CH ₂ -(R)Cha-(RorS)Pic(4,5-dehydro)-Nag/b |
| | 82 | HOOC-CH ₂ -(R)Cha-Pic(4-(S)Me)-Nag |
| | 83 | HOOC-CH ₂ -(R)Pic(4-(R)Me)-Nag |
| | 84 | HOOC-CH ₂ -(R)Cgl-Pic-Nag |
| | 85 | H-(R)Hoc-Pro-Nag |
| 15 | 86 | HOOC-CH ₂ -(R)Hoc-Pro-Nag |
| | 87 | HOOC-CH ₂ -(R)Hoc-Pic-Nag |
| | 88 | HOOC-CH ₂ -(R)Dph-Pic-Nag |
| | 89 | HOOC-CH ₂ -(R)Dch-Pic-Nag |
| | 90 | HOOC-CH ₂ -(R)Cha-Pro(5-(R,S)Me)-Nag |
| 20 | 91 | H-(R)Cha-Pic(4-(R)Me)-Nag |
| | 92 | HOOC-CH ₂ -(R)Cha-Pic(4-(R)Me)-Nag |
| | 93 | HOOC-CH ₂ -(R)Cha-Pic(6-(S)Me)-Nag |

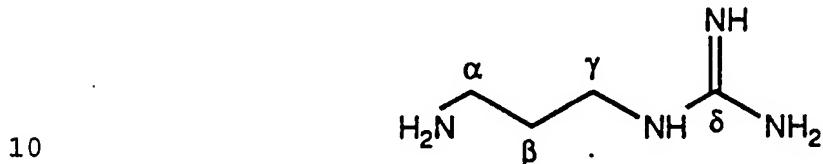
Of those compounds, the compounds having Example Nos. 4, 6,
25 9, 22, 30, 34, 59, 63, 67, 73, 80 and 82 are particularly
preferred, and of those the following compounds are most
preferred:

| | | |
|----|----|--|
| | 30 | HOOC-CH ₂ -(Me)(R)Cha-Pro-Nag |
| 30 | 34 | HOOC-(RorS)CH(Me)-(R)Cha-Pro-Nag/b |
| | 67 | HOOC-CH ₂ -(R)Cha-Pic-Nag |

35 In the above tables of compounds, the letters /a, /b, /c and
/d refer to a substantially pure stereoisomer at the carbon
atom denoted "RorS". The stereoisomer can be identified for

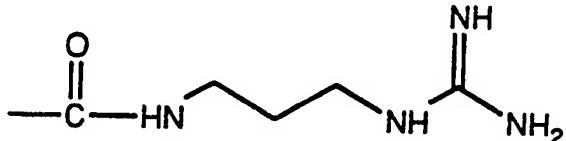
each compound with reference to the experimental part herein.
 "R,S" refers to a mixture of stereoisomers.

In a further embodiment the invention relates to novel use of
 5 a compound of the formula:



as a starting material in synthesis of a serine protease inhibitor, and in particular in synthesis of a thrombin 15 inhibitor. It can be used as such or having the guanidino group either mono protected at the δ -nitrogen or diprotected at the δ -nitrogens or the γ , δ -nitrogens, preferably with a protective group such as benzyloxy carbonyl. Protection of the noragmatine derivatives is carried out by methods known 20 in the art for guanidino compounds. This compound is named "noragmatine" or "Nag" herein. The compound has been previously disclosed inter alia as a hair bleaching accelerator in GB 1,599,324 (Henkel, priority date 5.2.1977).
 The structural fragment of the formula

25



30

has however not been previously disclosed as a structural element in a pharmaceutically active compound. As such structural element the "noragmatine" fragment renders a 35 serine protease inhibitor, and in particular a thrombin inhibitor valuable.

Medical and pharmaceutical use

In a further embodiment the invention relates to treatment, in a human or animal organism, of conditions where inhibition 5 of thrombin is required. The compounds of the invention are expected to be useful in particular in animals including man in treatment or prophylaxis of thrombosis and hypercoagulability in blood and tissues. It is furthermore expected to be useful in situations where there is an undesirable excess of 10 the thrombin without signs of hypercoagulability. Disease states in which the compounds have a potential utility, in treatment and/or prophylaxis, include venous thrombosis and pulmonary embolism, arterial thrombosis, such as in myocardial infarction, unstable angina, thrombosis-based 15 stroke and peripheral arterial thrombosis. Further, the compounds have expected utility in prophylaxis of atherosclerotic diseases such as coronary arterial disease, cerebral arterial disease and peripheral arterial disease. Further, the compounds are expected to be useful together 20 with thrombolytics in thrombotic diseases, in particular myocardial infarction. Further, the compounds have expected utility in prophylaxis for re-occlusion after thrombolysis, percutaneous trans-luminal angioplasty (PTCA) and coronary bypass operations. Further, the compounds have expected 25 utility in prevention of re-thrombosis after microsurgery. Further, the compounds are expected to be useful in anti-coagulant treatment in connection with artificial organs and cardiac valves. Further, the compounds have expected utility in anticoagulant treatment in haemodialysis and disseminated 30 intravascular coagulation.

A further expected utility is in rinsing of catheters and mechanical devices used in patients in vivo, and as an anticoagulant for preservation of blood, plasma and other 35 blood products in vitro.

Pharmaceutical preparations

The compounds of the Formula I will normally be administered by the oral, rectal, dermal, nasal or parenteral route in the 5 form of pharmaceutical preparations comprising the active ingredient either as a free base or a pharmaceutical acceptable non-toxic acid addition salt, e.g. the hydrochloride, hydrobromide, lactate, acetate, citrate, p-toluenesulfonate, trifluoroacetate and the like in a 10 pharmaceutically acceptable dosage form.

The dosage form may be a solid, semisolid or liquid preparation prepared by *per se* known techniques. Usually the active substance will constitute between 0.1 and 99 % by 15 weight of the preparation, more specifically between 0.1 and 50 % by weight for preparations intended for parenteral administration and between 0.2 and 75 % by weight for preparations suitable for oral administration.

20 Suitable daily doses of the compounds of the invention in therapeutical treatment of humans are about 0.001-100 mg/kg body weight at peroral administration and 0.001-50 mg/kg body weight at parenteral administration.

25

Preparation

A further objective of the invention is the mode of preparation of the compounds. The compounds of Formula I may be 30 prepared by coupling of an N-terminally protected amino acid or dipeptide or a preformed, N-terminally alkylated protected dipeptide to a compound



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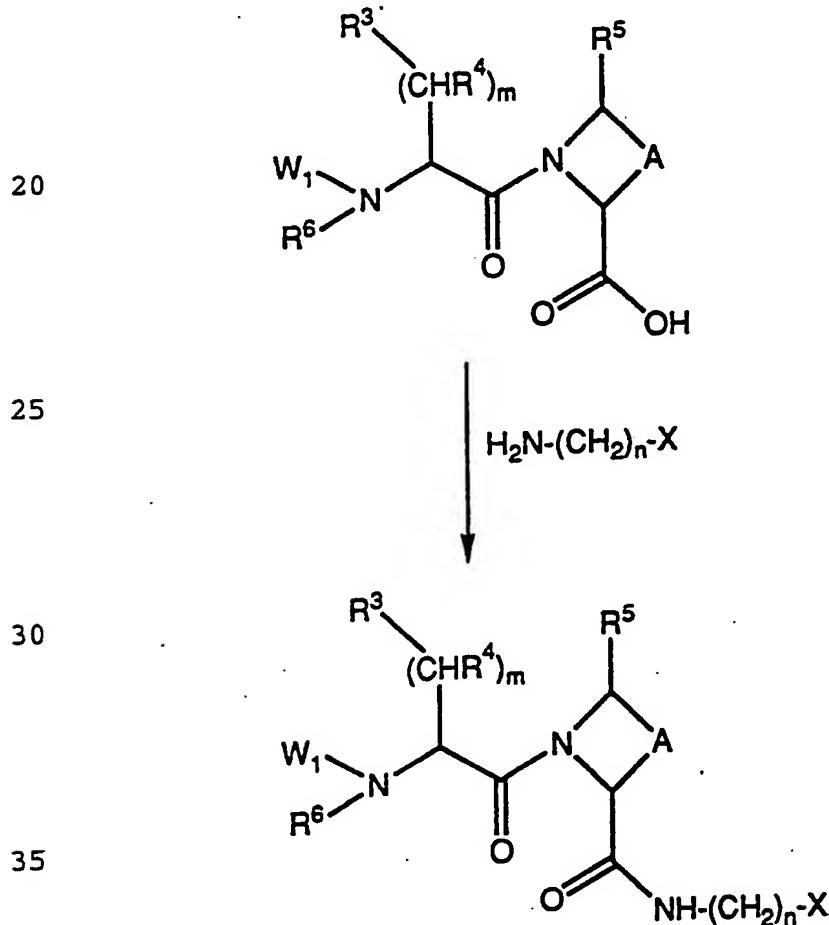
wherein n is as defined with Formula I and X is an unprotected or protected guanidino group or a protected amino

group, or a group transferable into an amino group, where the amino group is subsequently transferred into a guanidino group.

5 The coupling is accordingly done by one of the following methods:

Method I

10 Coupling of an N-terminally protected dipeptide, prepared by standard peptide coupling, with either a protected- or unprotected amino guanidine or a straight chain alkylamine carrying a protected or masked amino group at the terminal end of the alkyl chain, using standard peptide coupling, shown
15 in the formula



wherein R³, R⁴, R⁵, n, m and A are as defined in Formula I, R⁶ is H or alkyl, W₁ is an amino protecting group such as tertiarybutoxy carbonyl and benzyloxy carbonyl and X is -NH-C(NH)NH₂, -NH-C(NH)NH-W₂, -N(W₂)-C(NH)NH-W₂,

5 -NH-C(NW₂)NH-W₂ or -NH-W₂, where W₂ is an amine protecting group such as tertiarybutoxy carbonyl or benzyloxy carbonyl, or X is a masked amino group such as azide, giving the protected peptide. The final compounds can be made in any of the following ways, depending on the nature of the X- group

10 used: Removal of the protecting group(s) (when X= -NH-C(NH)NH₂, -N(W₂)-C(NH)NH-W₂, -NH-C(NW₂)NH-W₂ or -NH-C(NH)NH-W₂), or a selective deprotection of the W₁- group (e.g when X= -NH-C(NH)NH-W₂, -N(W₂)-C(NH)NH-W₂, -NH-C(NW₂)NH-W₂, W₂ in this case must be orthogonal to W₁) followed by

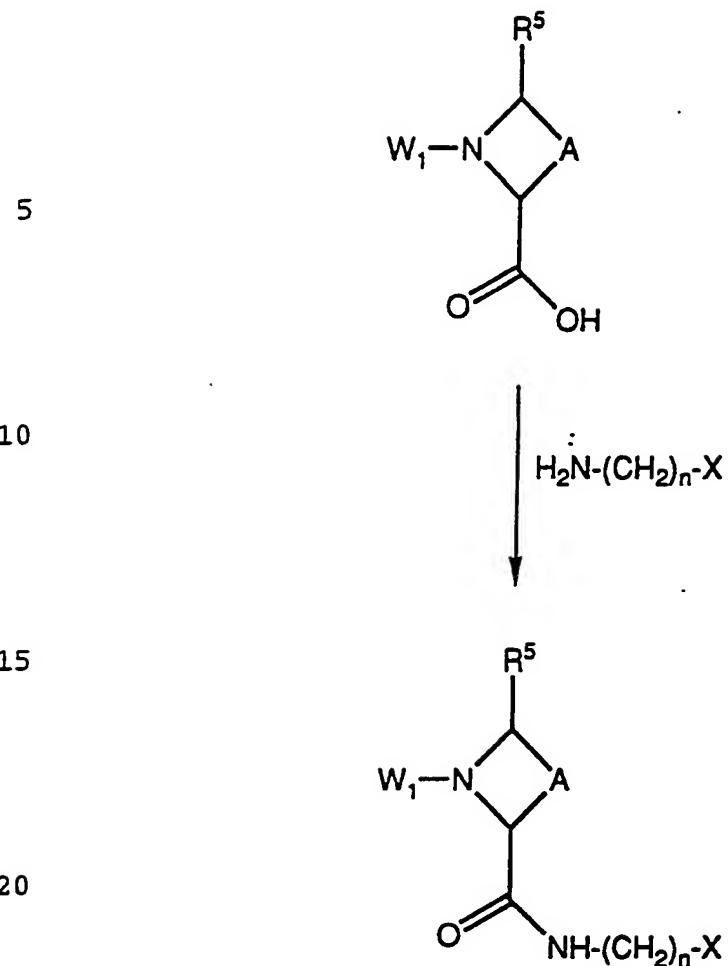
15 alkylation of the N-terminal nitrogen and deprotection or a selective deprotection/unmasking of the terminal alkylamino function (X= NH-W₂ , W₂ in this case must be orthogonal to W₁ or X= a masked aminogroup, such as azide) followed by a guanidation reaction, using standard methods, of the free

20 amine and deprotection of the W₁-group.

Method II

25 Coupling of an N-terminally protected amino acid, prepared by standard methods, with either a protected- or unprotected amino guanidine or a straight chain alkylamine carrying a protected or masked amino group at the terminal end of the alkyl chain, using standard peptide coupling, shown in the

30 formula

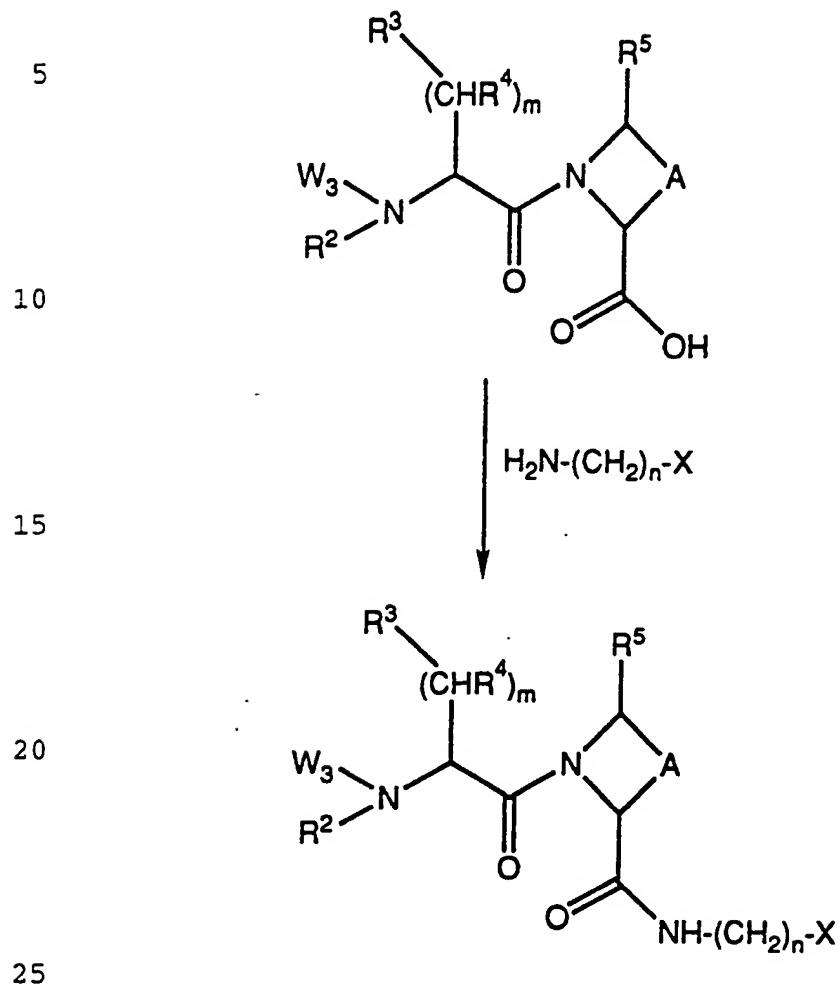


wherein W_1 , A, R^5 and X are as defined above followed by deprotection of the W_1 -group and coupling with the N-terminal 25 amino acid, in a protected form, leading to the protected peptide described in Method I or III, depending on the choice of the substitution pattern on the nitrogen of the N-terminal amino acid used in the coupling. The synthesis is then continued according to Method I or Method III to give the 30 final peptides.

Method III

Coupling of a preformed N-terminally alkylated and protected 35 dipeptide, prepared by standard peptide coupling, with either a protected or unprotected amino guanidine or a straight chain alkylamine carrying a protected or masked aminogroup at

the terminal end of the alkyl chain, using standard peptide coupling, shown in the formula



wherein R^2 , R^3 , R^4 , R^5 , n , m , A and X are defined as above provided that R^2 is other than H and W_3 is an acyl protecting group such as trifluoroacetyl.

30 The final compounds can be made in any of the following ways depending on the nature of the X -group used: Removal of protecting groups (when $X = NH-C(NH)NH_2$, $NH-C(NH)NH-W_2$, $N(W_2)-C(NH)NH-W_2$, $NH-C(NW_2)NH-W_2$ or $NH-W_2$) or a selective
35 deprotection/unmasking of the terminal alkylamino function ($X = NH-W_2$, W_2 in this case must be orthogonal to W_3 or $X =$ a masked amino group such as azide) followed by a guanidation

deprotection of the W₃ group.

DETAILED DESCRIPTION OF THE INVENTION

5 The following description is illustrative of aspects of the invention.

EXPERIMENTAL PART

10 Synthesis of the compounds of the invention is illustrated in Schemes I to VI appended hereto.

General Experimental Procedures.

15 The ¹H NMR and ¹³C NMR measurements were performed on BRUKER AC-P 300 and BRUKER AM 500 spectrometers, the former operating at a ¹H frequency of 500.14 MHz and a ¹³C frequency of 125.76 MHz and the latter at ¹H and ¹³C frequency of 300.13 MHz and 75.46 MHz respectively.

20 The samples were 10-50 mg dissolved in 0.6 ml of either of the following solvents; CDCl₃ (isotopic purity > 99.8%, Dr. Glaser AG Basel), CD₃OD (isotopic purity > 99.95%, Dr. Glaser AG Basel) or D₂O (isotopic purity > 99.98%, Dr. Glaser AG Basel).

The ¹H and ¹³C chemical shift values in CDCl₃ and CD₃OD are relative to tetramethylsilane as an external standard. The ¹H chemical shifts in D₂O are relative to the sodium salt of 30 3-(trimethylsilyl)-d₄-propanoic acid and the ¹³C chemical shifts in D₂O are referenced relative to 1,4-dioxane (67.3 ppm), both as external standard. Calibrating with an external standard may in some cases cause minor shift differences compared to an internal standard, however, the difference in 35 ¹H chemical shift is less than 0.02 ppm and in ¹³C less than 0.1 ppm.

The ^1H NMR spectrum of peptide sequences containing a proline residue frequently exhibits two sets of resonances. This corresponds to the existence of two contributing conformers with respect to the rotation around the amide bond, where 5 proline is the N-part of the amide bond. The conformers are named **cis** and **trans**. In our compounds the sequences (R)Cha-Pro- and -(R)Cha-Pic- often give rise to a cis-trans equilibrium with one conformer as the preponderant conformer (>90%). In those cases only the ^1H chemical shifts of the 10 major rotamer is reported.

Thin-Layer Chromatography was carried out on commercial Merck Silicagel 60F₂₅₄ coated glass or aluminium plates. Visualization was by a combination of UV-light, followed by spraying 15 with a solution prepared by mixing 372 ml of EtOH(95%), 13.8 ml of concentrated H₂SO₄, 4.2 ml of concentrated acetic acid and 10.2 ml of p-methoxy benzaldehyde or phosphomolybdic acid reagent (5-10 w.t % in EtOH(95%)) and heating.

20 Flash chromatography was carried out on Merck Silicagel 60 (40-63 mm, 230-400 mesh) under pressure of N₂.

Reversed phase high-performance liquid chromatography (in the Examples referred to as RPLC) was performed on a Waters M-590 25 instrument equipped with three reverse phase Kromasil 100,C8 columns (Eka-Nobel) having different dimensions for analytical (4.6 mm x 250 mm), semipreparative (1" x 250 mm) and preparative (2" x 500 mm) chromatography detecting at 226 nm.

30 Freeze-drying was done on a Leybold-Heraeus, model Lyovac GT 2, apparatus.

Protection Procedures**Boc-(R)Cha-OH**

5 To a solution of H-(R)Cha-OH, 21.55 g (125.8 mmol), in 130 ml 1 M NaOH and 65 ml THF was added 30 g (137.5 mmol) of $(\text{Boc})_2\text{O}$ and the mixture was stirred for 4.5 h at room temperature. The THF was evaporated and an additional 150 ml of water was added. The alkaline aqueous phase was washed twice with

10 EtOAc, then acidified with 2 M KHSO₄ and extracted with 3 x 150 ml of EtOAc. The combined organic phase was washed with water, brine and dried (Na_2SO_4). Evaporation of the solvent afforded 30.9 g (90.5 %) of the title compound as a white solid.

15

Z-(R)Cha-OH

The same procedure as described in Bodanszky M. and Bodanszky A. "The Practice of Peptide Synthesis", Springer-Verlag, 20 1984, p. 12, was used starting from H-(R)Cha-OH.

Boc-(Me)Phe-OH

Prepared in the same way as Boc-(R)Cha-OH from Me-(R)Phe-OH.

25

Boc-(R,S)Pro(3-(trans)Ph)-OH

To a well stirred solution of 2.0 g (8.8 mmol, 1 eq.) H-(R,S)Pro(3-(trans)Ph)-OH x HCl (Prepared as described in 30 J. Org. Chem., 55, p. 270-75, 1990 and J. Org. Chem., 39, 1710-1716, 1974), in 17.6 ml of 1 N NaOH, 12 ml of H₂O and 12 ml of THF at +5 °C was added 2.33 g $(\text{Boc})_2\text{O}$ (10.7 mmol, 1.2 eq.). The reaction was allowed to reach room temperature and the stirring was continued for an additional 18 h. The 35 organic solvent was evaporated and 50 ml of H₂O was added to the residue. The basic water phase was washed with 2x50 ml of EtOAc and acidified with 2 M KHSO₄ (pH about 1). The acidic

water phase was extracted with 4x75 ml of EtOAc and the combined organic phase was washed with 1x40 ml of H₂O, 1x40 ml of brine and dried (MgSO₄). Evaporation of the solvent gave 2.0 g (78 %) of pure product as a white solid.

5

¹H-NMR (CDCl₃, 500 MHz, mixture of two rotamers): δ 1.4 and 1.5 (2s, 9H), 2.0-2.1 (m, 1H), 2.3-2.4 (m, 1H), 3.45-3.88 (m, 3H), 4.3 and 4.45 (2d, 1H), 7.2-7.4 (m, 5H).

10 **Boc-(R,S)Pro(3-Ph)-OH**

Prepared as above starting from a cis/trans mixture of H-(R,S)Pro(3-Ph)-OH.

15 **Boc-(R)Dph-OH**

Prepared according to the method described by K. Hsich et.al. in J. Med. Chem., 32, p. 898 (1989) from H-(R)Dph-OH.

20

Boc-(R)Hop-OH

Prepared by the same procedure as described for Boc-(R)Cha-OH starting from H-(R)Hop-OH.

25

¹H-NMR (300 MHz, CDCl₃): δ 1.45 (s, 9H), 2.00 (m, 1H), 2.22 (m, 1H), 2.75 (bt, 2H), 4.36 (bs, 1H), 5.05 (bs, 1H), 7.15-7.33 (m, 5H).

30

Deprotection Procedures.

(a) The protected peptide was dissolved in EtOH (95%) and hydrogenated over 5 % Pd/C at atmospheric pressure in the presence of an excess of TFA or HOAc (> 2 eq.) for about 1-4 h. The catalyst was filtered off, the solvent evaporated and the final peptide (TFA or HOAc salt) was isolated as a

white powder after freeze drying (H_2O)

(b) The same as in (a) except that EtOH/ H_2O (ca:5/1) was used as solvent.

5

(c) The same procedure as in (a) but MeOH was used as solvent.

(d) The same procedure as in (a) but 2 M HCl was used as acid
10 to give the HCl-salt.

(e) Hydrolysis of esters, an illustrative example:

EtOOOC- CH_2 -(R)Cha-Pro-Nag x 2 HOAc (0.4 mmol) was dissolved in
15 1.5 ml of MeOH and 1.2 ml (1.2 mmol) of 1M NaOH was added at room temperature. After 3 h the methanol was evaporated and an excess HOAc was added to the residue and the mixture was freeze dried and purified by RPLC ($\text{CH}_3\text{CN}/0.1$ M NH_4OAc (70/30)). The pure product was obtained as a powder in 73 %
20 yield after freeze drying from water.

(f) Cleavage of t-butyl esters, an illustrative example:

The t-butyl ester was dissolved in an excess of TFA. After
25 stirring for 2 h at room temperature the TFA was evaporated. Purification by treatment with activated charcoal in water-ethanol was followed by freeze drying from water giving the desired compounds.

30 Preparation of Starting Materials.

H-Pic-OEt x HCl

L-Pipecolinic acid, 4.0 g (0.031 mol), was slurried in 100 ml
35 of abs. ethanol and HCl (g) was briefly bubbled through until a clear solution was obtained. It was cooled in an ice bath and 17 ml of thionyl chloride was added dropwise over 15 min.

The ice bath was removed and the mixture was refluxed for 2.5 h. The solvent was evaporated and the product was obtained as its hydrochloride salt in a quantitative yield.

5 ¹H-NMR (300 MHz, D₂O): δ 1.33 (t, 3H), 1.8-2.1 (m, 5H), 2.3-2.5 (m, 1H), 3.1-3.3 (m, 1H), 3.5-3.7 (m, 1H), 4.14 (dd, 1H), 4.44 (q, 2H).

H-Pic-OMe × HCl

10

Prepared in the same way as described for H-Pic-OEt × HCl by replacing EtOH with MeOH.

H-Aze-OEt × HCl

15

Prepared in the same way as described for H-Pic-OEt × HCl from H-Aze-OH.

H-Pic(4-(S)Me)-OEt × HCl

20

Prepared in the same way as described for H-Pic-OEt × HCl from H-Pic(4-(S)Me)-OH (purchased from Synthelec, Lund, Sweden).

25 **H-(R)Pic(4-(R)Me)-OEt × HCl**

Prepared in the same way as described for H-Pic-OEt × HCl from H-(R)Pic(4-(R)Me)OH (purchased from Synthelec, Lund, Sweden).

30

H-(R)Dph-OH

Prepared by the general method given by A. Evans et. al. in JACS, 112, 4011 (1990).

35

H-(R,S)Pic(4,5-dehydro)-OEt

H-(R,S)Pic(4,5-dehydro)-OH, 3.05 g (18.1 mmol) (Prepared according to the procedure by Burgstahler et. al. J. Org. Chem., 25, 4, p. 489-92 (1960), was dissolved in 75 ml EtOH/HCl (saturated) and the mixture was refluxed for 5 hours. The solvent was evaporated and the remaining residue was dissolved in water, made alkaline with sodium hydroxide (aq) and extracted three times with ethylacetate. Drying (Na_2SO_4) and carefull evaporation gave 2,05g (71%) of the title compound.

¹⁰ $^1\text{H-NMR}$ (CDCl_3): δ 1.28 (t, 3H), 1.88 (bs, NH) 2.2-2.4 (m, 2H), 3.45 (bs, 2H), 3.57 (dd, 1H), 4.21 (q, 2H), 5.68-5.82 (m, 2H).

¹⁵ Boc-(R)Cgl-OH

Boc-(R)Pgl-OH was hydrogenated over 5% Rh/ Al_2O_3 in MeOH at 5 Mpa. Filtration and evaporation of the solvent gave the title compound which was used without further purification.

²⁰ $^1\text{H-NMR}$ (300 MHz, CDCl_3): δ 0.9-1.7 (m, 20H), 4.0-4.2 (m, 1H), 5.2 (d, 1H).

Boc-(R)Dch-OH

²⁵ Boc-(R)Dph-OH, 0.75 g (2.2 mmol), was dissolved in 25 ml of MeOH and a catalytic amount of 5% Rh/ Al_2O_3 was added. The mixture was hydrogenated at 5 Mpa, 50°C for 40 h, filtered and evaporated to give 0.72 g (93%) of the thitle compound.

³⁰ $^1\text{H-NMR}$ (CDCl_3): δ 0.9-2.0 (m, 32H), thereof 1.45 (bs, 9H), 4.55 (bd) and 4.9 (bd); two rotamers integrating for a total of 1H, 5.7-6.1 (broad, NH).

³⁵ H-(R)Pro(5-(S)Me)-OMe

Prepared according to the procedure given by B. Gopalan et.al. in J. Org. Chem., 51, 2405, (1986).

H-Mor-OH

Prepared according to the method of K. Nakajima. et al. Bull. Chem. Soc. Jpn., 51 (5), 1577-78, 1978 and ibid 60, 5 2963-2965, 1987.

H-Mor-OEt x HCl

Prepared in the same way as H-Pic-OEt x HCl from H-Mor-OH.
10

Boc-(R)Cha-OSu

Boc-(R)Cha-OH (1 eq.), HOSu (1.1 eq) and DCC or CME-CDI (1.1 eq) were dissolved in acetonitrile (about 2.5 ml/mmol acid)
15 and stirred at room temperature over night. The precipitate formed during the reaction was filtered off, the solvent evaporated and the product dried in vacuo. (When CME-CDI was used in the reaction the residue, after evaporation of the CH₃CN, was dissolved in EtOAc and the organic phase washed
20 with water and dried. Evaporation of the solvent gave the title compound).

¹H-NMR (500 MHz, CDCl₃, 2 rotamers ca: 1:1 ratio) δ 0.85-1.1 (m, 2H), 1.1-1.48 (m, 4H), 1.5-1.98 (m, 16H; thereof 1.55
25 (bs, 9H)), 2.82 (bs, 4H), 4.72 (bs, 1H, major rotamer), 4.85 (bs, 1H, minor).

Boc-(Me)(R)Cha-OSu

30 (i) Boc-(Me)(R)Cha-OH

A solution of 11.9 g (42.6 mmol) Boc-(Me)(R)Phe-OH in 150 ml MeOH was hydrogenated over 5% Rh/Al₂O₃ at 0,28 Mpa for 24 h. Filtration of the catalyst and evaporation of the solvent
35 gave the product as a white solid (95 % yield) which was used in the next step without further purification.

1H-NMR (500 MHz, CDCl₃, mixture of two rotamers ca: 1/1). δ 0.8-1.1 (m, 2H), 1.1-1.9 (m, 20H, thereof 1.47 and 1.45 (s, 9H)), 2.82 and 2.79 (s, total 3H), 4.88 and 4.67 (m, total 1H).

5

(ii) Boc-(Me)(R)Cha-OSu

Prepared in the same way as described for Boc-(R)Cha-OSu- from Boc-(Me)(R)Cha-OH.

10

Boc-(R)Cha-Pro-OSu

(i) Boc-(R)Cha-Pro-OH

15 H-(S)Pro-OH (680 mmol) was dissolved in 0.87M sodium hydroxide (750 ml). Boc-(R)Cha-OSu (170 mmol) dissolved in DMF (375 ml) was added dropwise during 20 min. The reaction mixture was stirred at room temperature for 20 h. The mixture was acidified (2M KHSO₄) and extracted three times with ethyl acetate. The organic layers were combined and washed three times with water and once with brine. After drying over sodium sulphate and evaporation of the solvent, the syrupy oil was dissolved in diethyl ether, the solvent evaporated and finally the product dried in vacuo to yield

20 Boc-(R)Cha-Pro-OH as a white powder in almost quantitative yield.

25

1H-NMR (500 MHz, CDCl₃, minor rotamer 10%) δ 0.8-1.05 (m, 2H), 1.05-1.55 (m, 15H; thereof 1.5 (bs, 9H)), 1.55-1.8 (m, 5H), 1.8-2.15 (m, 3H), 2.47 (m, 1H), 3.48 (m, 1H), 3.89 (m, 1H), 4.55 (m, 2H), 5.06 (m, 1H); minor rotamer signals 2.27 (m, 1H), 3.58 (m, 1H), 4.33 (m, 1H), 5.0 (m, 1H)

(ii) Boc-(R)Cha-Pro-OSu

35

Prepared in the same way as described for Boc-(R)Cha-OSu- from Boc-(R)Cha-Pro-OH.

¹H-NMR (500 MHz, CDCl₃, 2 rotamers, 5:1 ratio) δ 0.78-1.05 (m, 2H), 1.05-1.83 (m, 20H; thereof 1.43 (bs, 9H)), 1.83-2.26 (m, 3H), 2.32 (m, 1H), 2.72-2.9 (m, 4H), 3.2 (m, 1H, minor rotamer), 3.52 (m, 1H, major), 3.68 (m, 1H, minor rotamer), 5 3.89 (m, 1H, major), 4.31 (bq, 1H, minor rotamer), 4.56 (bq, 1H, major), 4.71 (bt, 1H, major rotamer), 4.93 (bt, 1H, minor), 5.22 (bd, 1H, major rotamer), 5.44 (bd, 1H, minor).

10 Z-(R)Cha-Pro-OSu

Prepared in the same way as Boc-(R)Cha-Pro-OSu from Z-(R)Cha-OH.

15 Boc-(R)Cha-Pic-OSu

(i) Boc-(R)Cha-Pic-OEt

Boc-(R)Cha-OH, 6.3 g (0.023 mol), was dissolved in 150 ml of 20 CH₂Cl₂. The solution was cooled in an ice bath and 6.3 g (0.047 mol) of N-hydroxybenzotriazole and 11.2 g (0.0265 mol) of CME-CDI were added. The ice bath was removed after 15 min and the reaction mixture was stirred for 4 h at room temperature. The solvent was evaporated and the residue dissolved in 25 150 ml of DMF and cooled in an ice bath. H-Pic-OEt_xHCl, 4.1 g (0.021 mol) was added and the pH adjusted to approximately 9 by addition of N-methylmorpholine. The ice bath was removed after 15 min and the reaction mixture was stirred for 3 days. The solvent was evaporated and the residue was dissolved in 30 ethyl acetate and washed with dilute KHSO₄ (aq), NaHCO₃ (aq) and water. The organic layer was dried (Na₂SO₄) and evaporated to give 7.7 g (89 %) of Boc-(R)Cha-Pic-OEt which was used without further purification.

35 ¹H-NMR (500 MHz, CDCl₃, 2 rotamers, 3:1 ratio) δ 0.7-1.0 (m, 2H), 1.1-1.9 (m, 29H; thereof 1.28 (t, 3H)), 1.45 (bs, 9H), 2.01 (bd, 1H, major rotamer), 2.31 (bd, 1H), 2.88 (bt, 1H,

minor), 3.30 (bt, 1H, major), 3.80 (bd, 1H, major), 4.15-4.3 (m, 2H), 4.5-4.7 (m, 2H, minor), 4.77 (bq, 1H, major), 4.90 (bd, 1H, minor), 5.28 (bd, 1H, major), 5.33 (bd, 1H, major).

5 (ii) Boc-(R)Cha-Pic-OH

Boc-(R)Cha-Pic-OEt, 5.6 g (0.014 mol), was mixed with 100 ml of THF, 100 ml of water and 7 g of LiOH. The mixture was stirred at room temperature overnight. The THF was evaporated 10 and the aqueous solution was acidified with KHSO₄ (aq) and extracted three times with ethyl acetate. The combined organic phase was washed with water, dried (Na₂SO₄) and evaporated to give 4.9 g (94 %) of Boc-(R)Cha-Pic-OH which was used without further purification. The compound can be 15 crystallized from diisopropyl ether/hexane.

¹H-NMR (500 MHz, CDCl₃, 2 rotamers, 3.5:1 ratio) δ 0.8-1.1 (m, 2H), 1.1-2.1 (m, 27H; thereof 1.43 (s, 9H, major rotamer), 1.46 (s, 9H, minor)), 2.33 (bd, 1H), 2.80 (bt, 1H, minor), 3.33 (bt, 1H, major), 3.85 (bd, 1H, major), 4.57 (bd, 1H, minor), 4.68 (m, 1H, minor), 4.77 (bq, 1H, major), 5.03 (bs, 1H, minor), 5.33 (bd, 1H, major), 5.56 (m, 1H, major).

25 (iii) Boc-(R)Cha-Pic-OSu

Boc-(R)Cha-Pic-OH (1 g, 2.6 mmol) was dissolved in DMF (15 ml) at room temperature and then cooled to - 18°C, a temperature which was maintained during the additions of the 30 reactants. Hydroxy succinimid (0.60 g, 5.2 mmol) was added and the reaction mixture was stirred for a few minutes until the crystals were dissolved. Dicyclohexyl carbodiimid (0.56 g, 2.7 mmol) dissolved in DMF (10 ml) and precooled was added dropwise to the rection mixture. After a few minutes at -18°C 35 the reaction mixture was put into a water bath at 20°C for 2 h under stirring. The solvent was evaporated, ethyl acetate (40 ml) was added and the precipitated urea was filtered off.

The organic phase was washed once with water, twice with 0.3 M KHSO₄, twice with diluted NaHCO₃, once with water, once with brine and dried (Na₂SO₄). The solvent was evaporated and the product dried in vacuo to yield 1.16 g (93%) of the 5 product. According to ¹H-NMR the product contained two diastereoisomers (epimers in Pic, S/R) in a ratio of 95/5.

¹H-NMR (300 MHz, CDCl₃, major diastereomer) δ 0.7-2.0 (m, 27H; thereof 1.46 (bs, 9H)), 2.29 (bd, 1H), 2.85 (bs, 4H), 10 3.40 (m, 1H), 4.5-4.8 (m, 1H), 5.1-5.4 (m, 1H), 5.70 (bd, 1H, major).

Boc-(R)Cha-Mor-OSu

15 Prepared in the same way as Boc-(R)Cha-Pic-OSu from H-Mor-OEt × HCl except that CH₃CN was used as solvent insted of DMF in the formation of the OSu-ester.

Boc-(Me)(R)Cha-Pro-OSu

20 Prepared in the same way as Boc-(R)Cha-Pro-OSu from Boc-(Me)-(R)Cha-OH.

Boc-(Me)(R)Cha-Pic-OSu

25 Prepared in the same way as Boc-(R)Cha-Pic-OSu from Boc-(Me)(R)Cha-OH.

Boc-(R,S)Pro(3-Ph)-Pro-OSu

30 Prepared in the same way as Boc-(R)Cha-Pro-OSu from Boc-(R,S)Pro(3-Ph)-OH.

Boc-(R,S)Pro(3-(trans)Ph)-Pro-OSu

35 (i) Boc-(R,S)Pro(3-(trans)Ph)-Pro-OBn

To a slurry of 1.0 g of Boc-(R,S)Pro(3-(trans)Ph)-OH (3.43 mmol, 1 eq.), 1.04 g of H-Pro-OBn x HCl (4.29 mmol, 1.25 eq.), 0.04 g of HOEt (0.24 mmol, 0.07 eq.) in 15 ml DMF was added 1.83 g of CME-CDI (4.29 mmol, 1.25 eq.) and 0.525 ml of 5 NMM (4.73 mmol, 1.38 eq.) at room temperature. After stirring an additional 4 days the solvent was evaporated and the residue taken up in 200 ml EtOAc. The organic phase was washed with 2x40 ml of H₂O, 2x25 ml of 1M KHSO₄, 2x25 ml of 1M NaOH, 2x25 ml of H₂O and dried (MgSO₄). Evaporation of the 10 solvent and flash chromatography (CH₂Cl₂/MeOH, 97/3) gave the pure product (44% yield) as a ca: 1:1 mixture of diastereomers.

(ii) Boc-(R,S)Pro(3-(trans)Ph)-Pro-OH

15 The benzyl ester from the previous step was removed by hydrogenation over 5 % Pd/C in EtOH at atmospheric pressure for 4 h. Filtration and evaporation gave the pure product as a ca: 1:1 mixture of diastereomers in quantitative yield.

20 ¹H-NMR (CDCl₃, 500 MHz, two diastereomers each consisting of two rotamers): δ 1.3-2.4 (m + 4s from the Boc groups, total 14H), 2.5-2.9 (m, total 1H), 3.2-3.9 (m, total 5H), 4.3-4.65 (m, total 2H), 7.2-7.5 (m, 5H).

25 (iii) Boc-(R,S)Pro(3-(trans)Ph)-Pro-OSu

Prepared according to the procedure described for Boc-(R)Cha-OSu from Boc-(R,S)Pro(3-(trans)Ph)-Pro-OH.

30 Boc-(R,S)Pro(3-(trans)Ch)-Pro-OSu

(i) Boc-(R,S)Pro(3-(trans)Ch)-Pro-OH

35 Boc-(R,S)Pro(3-(trans)Ph)-Pro-OH was hydrogenated over 5 % Rh/Al₂O₃ in methanol together with a small amount of HOAc for 7 days at 0,34 Mpa. Filtration of the catalyst, evaporation

of the solvent and flash chromatography ($\text{CH}_2\text{Cl}_2/\text{MeOH}$, 94:6) gave the pure product as a white solid (mixture of two diastereomers).

5 (ii) **Boc-(R,S)Pro(3-(trans)Ch)-Pro-OSu**

Prepared according to the procedure described for **Boc-(R)Cha-OSu** from **Boc-(R,S)Pro(3-(trans)Ch)-Pro-OH**.

10 **Boc-(R)Hoc-Pro-OH**

(i) **Boc-(R)Hoc-OH**

15 **Boc-(R)Hoc-OH**, 3.2 g (11.46 mmol) was dissolved in methanol (75 ml). Rhodium on activated aluminium oxide ($\text{Rh}/\text{Al}_2\text{O}_3$), 0.5 g was added and the mixture stirred in hydrogen atmosphere at 0.41 MPa for 18 h. The catalyst was filtered off through celite and the solvent evaporated giving the product in almost quantitative yield.

20 $^1\text{H-NMR}$ (500 MHz, CDCl_3): δ 0.90 (m, 2H), 1.08-1.33 (m, 6H), 1.43 (s, 9H), 1.60-1.74 (m, 6H), 1.88 (bs, 1H), 4.27 (bs, 1H).

25 (ii) **Boc-(R)Hoc-OSu**

Prepared in the same way as described for **Boc-(R)Cha-OSu** from **Boc-(R)Hoc-OH**.

30 (iii) **Boc-(R)Hoc-Pro-OH**

Prepared in the same way as described for **Boc-(R)Cha-Pro-OH** from **Boc-(R)Hoc-OSu**.

35 $^1\text{H-NMR}$ (500 MHz, CDCl_3): δ 0.80-0.94 (m, 2H), 1.05-1.36 (m, 7H), 1.36-1.48 (bs, 9H), 1.48-1.78 (m, 7H), 1.98-2.14 (m, 2H), 2.34 (m, 1H), 3.48 (m, 1H), 3.85 (m, 1H), 4.43 (m, 1H),

4.52 (bd, 1H), 5.26 (bd, 1H), signals of a minor rotamer appears at: δ 1.92, 2.25, 3.58, 4.20 and 4.93.

Boc-(R)Hoc-Pic-OH

5

(i) **Boc-(R)Hoc-Pic-OMe**

Prepared the same way as described for Boc-(R)Cha-Pic-OEt from Boc-(R)Hoc-OH and H-Pic-OMe x HCl.

10 (ii) **Boc-(R)Hoc-Pic-OH**

Prepared in the same way as described for Boc-(R)Cha-Pic-OH from Boc-(R)Hoc-Pic-OMe.

15 $^1\text{H-NMR}$ (500 MHz, CDCl_3): δ 0.82-0.97 (m, 2H), 1.10-1.36 (m, 7H), 1.36-1.50 (bs, 9H), 1.50-1.82 (m, 11H), 2.35 (bd, 1H) 3.28 (bt. 1H), 3.85 (bd, 1H) 4.63 (m, 1H), 5.33 (bs, 1H), 5.44 (bd, 1H), signals of a minor rotameter appears at: δ 1.88, 2.80, 4.25, 4.55 and 4.97.

20

Boc-(R)Cha-Aze-OH

Prepared in the same way as described for Boc-(R)Cha-Pic-OH from H-Aze-OEt X HCl.

25

Boc-(R)Cha-Pic(4-(S)Me)-OH

Prepared in the same way as described for Boc-(R)Cha-Pic-OH from H-Pic(4-(S)Me)-OEt x HCl except that CH_2Cl_2 was used as 30 solvent.

Boc-(R)Cha-(R)Pic(4-(R)Me)-OSu

(i) **Boc-(R)Cha-(R)Pic(4-(R)Me)-OEt**

35

Prepared in the same way as described for Boc-(R)Cha-Pic-OEt from H-(R)Pic(4-(R)Me)-OEt x HCl.

(ii) Boc-(R)Cha-(R)Pic(4-(R)Me)-OH

Prepared by using the deprotection (e) on the product (i) above.

5

(iii) Boc-(R)Cha-(R)Pic(4-(R)Me)OSu

Prepared in the same way as described for Boc-(R)Cha-Pic-OSu from Boc-(R)Cha-(R)Pic(4-(R)Me)-OH.

10

Boc-(R)Cha-(R,S)Pic(4,5-dehydro)-OH

Prepared according to the procedure described for Boc-(R)Cha-15 Pic-OH from H-(R,S)Pic(4,5-dehydro)-OEt.

Boc-(R)Cgl-Pic-OH

(i) Boc-(R)Cgl-Pic-OMe

20

Pivaloyl chloride (1.000 mL, 8.1 mmol) was added to a solution of Boc-(R)Cgl-OH (2.086 g, 8.1 mmol) and triethyl amine (1.13 mL, 8.1 mmol) in toluene (25 mL) and DMF (5 mL). A mixture of H-Pic-OMe x HCl (1.46 g, 8.1 mmol) and 25 triethyl amine (1.13 mL, 8.1 mmol) in DMF (20 mL) was subsequently added at ice bath temperature. The reaction mixture was slowly allowed to warm up to room temperature and after 24 h it was diluted with water and extracted with toluene. After washing with 0.3 M KHSO₄, 10% Na₂CO₃ and brine 30 the solvent was removed in vacuo to give 2.52 g (81%) of colorless oil which was used without further purification.

¹H-NMR (500 MHz, CDCl₃, 2 rotamers, 5:1 ratio) δ 0.8-1.8 (m, 25H), 2.25 (d, 1H), 2.75 (t, 1H, minor rotamer), 3.3 (t, 1H), 35 3.7 (s, 3H), 3.85 (d, 1H), 4.3 (t, 1H, minor rotamer), 4.5-4.6 (m, 1H), 5.25 (d, 1H), 5.30 (d, 1H).

(ii) Boc-(R)Cgl-Pic-OH

Prepared according to the procedure for hydrolysis of Boc-(R)Cha-Pic-OEt using the product from (i) above. The product
5 was crystallized from di-isopropyl ether and hexane.

¹H-NMR (500 MHz, CDCl₃, 2 rotamers, 5:1 ratio) δ 0.8-1.8 (m,
25H), 2.3 (d, 1H), 2.8 (t, 1H, minor rotamer), 3.3 (t, 1H),
3.9 (d, 1H), 4.4 (t, 1H, minor), 4.5-4.6 (m, 1H), 5.1 (s, 1H,
10 minor rotamer), 5.3 (d, 1H), 5.40 (d, 1H).

Boc-(R)Dph-Pic-OH

Prepared in the same way as described for Boc-(R)Cha-Pic-OH
15 from Boc-(R)Dph-OH.

Boc-(R)Dch-Pic-OH

Prepared in the same way as described for Boc-(R)Cha-Pic-OH
20 from Boc-(R)Dch-OH.

Boc-(R)Cha-Pro(5-(S)Me)-OH

Prepared in the same way as described for Boc-(R)Cha-Pic-OH
25 from H-Pro(5-(S)Me)-OMe.

Boc-Nag(Z)

(i) N-Bensyloxycarbonyl-O-methyl isourea

30 To a stirred solution of concentrated aqueous NaOH (2.8 L,
50% w/w, 19.1 M, 53 mol) and water (32 L) at 18° C was added
in two portions O-methylisourea hemisulphate (1.7 kg, 94%,
13.0 mol) and O-methylisourea hydrogensulphate (1.57 kg, 99%,
35 9.0 mol). The reaction mixture was cooled to 3-5° C. Benzyl
chloroformate (3.88 kg, 92%, 20.9 mol) was added over a 20
minutes period under cooling and vigorous stirring. The

reaction temperature went from 3 to 8° C during the addition of Z-Cl. The addition funnel was rinsed with 5 litres of water which was added to the reactor. The reaction mixture was stirred at 0-3° C for 18 h, filtered and 5 the crystals was washed with cooled (3° C) water (10 L). Vacuum drying 25° C, 10-20 mbar) for 48 h gave 3.87 kg (89%) of the title compound as a white crystalline powder.

10 (ii) Boc-Nag(Z)

To a stirred solution Boc-NH-(CH₂)₃-NH₂ × HCl (prepared according to Mattingly P.G., Synthesis, 367 (1990)) (3.9 kg, 18.5 mol) in iso-propanol (24 kg) at 60-70° C was added in 15 portions over a 30 minutes period KHCO₃ (4.2 kg, 42 mol). A slow evolution of CO₂ (g) occurs. The mixture was stirred for another 30 minutes followed by addition in portions over a 30 minutes period N-bensyloxycarbonyl-O-methyl isourea (3.74 kg, 18.0 mol). The reaction mixture was stirred at 65-70° C for 20 16 h, cooled to 20° C and filtered. The precipitate was washed with iso-propanol (10 + 5 L). The combined filtrates was concentrated at reduced pressure keeping the heating mantle not warmer than 65-70° C. When approximately 45 litres was distilled off EtOAc (90 L) was added. The reaction 25 mixture was cooled to 20-25° C, washed with water (10 and 5 L) and brine (5 L), and dried with Na₂SO₄ (2 kg). After stirring the rection mixture was filtered and the filter cake was washed with EtOAc (11 and 7 L). The combined filtrates were concentrated at reduced 30 pressure keeping the heating mantle not warmer than 40-50° C. When approximately 90 litres of EtOAc was distilled off, toluene (25 L) was added and the evaporation continued. After collection of approximately another 18 litres of destillate, toluene (20 L) was added under vigorous stirring 35 and the resulting mixture was cooled to -1 to 0° C and gently stirred over night (17 h). The crystal slurry was filtered and the product was washed with cooled toluene (10 and 5 L).

vacuum drying (10-20 mbar,
40° C) for 24 h gave 4.83 kg (13.8 mol, 76%) of Boc-Nag(Z).

¹H-NMR (300 MHz, CDCl₃): δ 1.41 (s, 9H), 1.6-1.7 (m, 2H),
5 3.0-3.3 (m, 4H), 4.8-5.0 (bs, 1H), 5.10 (s, 2H), 7.2-7.4 (m,
5H).

Boc-Agm(Z)

10 (i) Boc-Agm

To a slurry of 14.95 g (65.5 mmol, 1 eq.) of agmatine sulphate (Aldrich), 13.7 ml of Et₃N (98.25 mmol, 1.5 eq.), 165 ml of H₂O and 165 ml of THF was added 21.5 g (98.25 mmol, 15 1.5 eq.) of (Boc)₂O during 5 minutes at room temperature. The mixture was stirred vigorously over night, evaporated to dryness and the residue was washed with 2x100 ml of Et₂O to give Boc-Agm as a white powder which was used without further purification in the next step.

20

(ii) Boc-Agm(Z)

To a cold (+5°C) slurry of the crude Boc-Agm from the previous step (ca: 65.5 mmol) in 180 ml of 4N NaOH and 165 ml 25 of THF was added 24 ml (169 mmol, 2.5 eq) of benzyl chloroformate during 10 minutes. After stirring at room temperature for 4 h methanol (150 ml) was added and the stirring was continued for an additional 20 h at room temperature. The organic solvent was evaporated and 200 ml of 30 H₂O was added to the residue. The basic water phase was extracted with 1x300 ml and 2x200 ml of EtOAc. The combined organic phases was washed with H₂O (2x100ml), brine (1x100 ml) and dried (MgSO₄). Evaporation of the solvent and flash chromatography (CH₂Cl₂/MeOH, a stepwise gradient of 97/3, 35 95/5 and 9/1 was used) gave 14.63 g (58%) of pure Boc-Agm(Z) as a white powder.

¹H-NMR (CDCl₃, 500 MHz): δ 1.35-1.40 (m, 2H), 1.45 (s, 9H), 1.5-1.6 (m, 2H), 3.0-3.2 (m, 4H), 4.65 (bs, 1H), 5.1 (s, 2H), 7.25-7.40 (m, 5H).

5 ¹³C-NMR (CDCl₃, 75.5 MHz): δ 25.44, 27.36, 28.21, 65.83, 79.15, 127.47, 127.66, 128.14, 137.29, 156.47, 161.48, 163.30.

Boc-NH-(CH₂)₃-N₃

10

Prepared according to the method described by Mattingly P. G., in Synthesis 1990, 367.

Z-NH-(CH₂)₂-NH₂

15

To a cold solution of 6 g ethylene diamine (0.1 mol) and 22 ml triethyl amine in 20 ml of chloroform was added 2.5 g of Z-OSu dissolved in 5 ml of chloroform. The mixture was allowed to reach room temperature and left over night under 20 stirring. Filtration, evaporation of the solvent and flash chromatography (CH₂Cl₂/MeOH(NH₃-saturated), 95/5) gave 0.9 g (46 %) of the title compound.

25 ¹H-NMR (300 MHz, CDCl₃): δ 1.27 (s, 2H), 2.85 (t, 2H), 3.24 (q, 2H), 5.14 (s, 2H), 7.22-7.40 (m, 5H).

Agm x HCl

30 Prepared from Agm x H₂SO₄ (Aldrich) by exchanging the hydrogen sulphate ion for chloride on an ion exchange column.

H-Nag(Z) x 2 HCl

35 Prepared by bubbling HCl(e) into a solution of Boc-Nag(Z) in EtOAc followed by evaporation of the solvent.

BnOOC-CH₂-NH-CO-CH₂-Br

To a solution of p-TsOH x H-Gly-OBn (5 mmol) and triethyl amine (5 mmol) in 10 ml of CH₂Cl₂ was added 2-bromoacetic acid (5 mmol) dissolved in 10 ml of CH₂Cl₂ and dicyclohexyl carbodiimide (5 mmol). The mixture was stirred at room temperature over night and filtered. The organic phase was washed twice with 0.2 M KHSO₄, 0.2 M NaOH, brine and dried. Evaporation and flash chromatography (CH₂Cl₂/MeOH, 95/5) gave 10 a quantitative yield of the desired compound.

¹H-NMR (300 MHz, CDCl₃) : δ = 3.89 (s, 2H); 4.05-4.11 (d, 2H), 5.19 (s, 2H), 7.06 (bs, 1H), 7.3-7.4 (m, 5H).

15 BnOOC-CH₂-OCO-CH₂-Br

A mixture of 2.8 g (0.020 mmol) bromoacetic acid, 4.2 g (0.020 mmol) of benzyl bromoacetate and 2.0 g (0.020 mmol) of triethylamine in 25 ml of EtOAc was refluxed for 3 h. It was 20 diluted with more EtOAc and cooled. The solution was washed with dilute HCl and thereafter with NaHCO₃ (aq) and finally with water. Drying (Na₂SO₄) and evaporation followed by flash chromatography (heptane/ethylacetate, 75/25) gave the title compound in 26 % yield.

25 ¹H-NMR (500 MHz, CDCl₃): δ 3.95 (s, 2H), 4.75 (s, 2H), 5.23 (s, 2H), 7.35-7.45 (m, 5H).

BnO-(CH₂)₃-OTf

30 Propanediol monobenzyl ether (0.83 g, 5 mmol) was dissolved in dry pyridine (0.6 g, 7 mmol) and dichloromethane (20 ml) and cooled to -15°C. Triflic anhydride, precooled to -15°C, was added and the reaction mixture stirred for 45 min under 35 which the temperature was allowed to rise to 15°C. The solvent was evaporated and the product dissolved in hexane/ethyl acetate 4:1 (10 ml) and filtered through silica.

Finally the solvent was evaporated and the product dried in vacuo to yield 0.95 g (64%) of 1-benzyloxy 3-trifluoromethanesulfonylpropane which was used directly (see Example 21).

5

$^1\text{H-NMR}$ (500 MHz, CDCl_3): δ 2.12 (m, 2H), 3.6 (t, 2H), 4.51 (s, 2H), 4.72 (t, 2H), 7.22-7.42 (m, 5H).

BnO-(CH₂)₂-CHO

10

Prepared by Swern oxidation (described by D. Swern et al., J. Org. Chem., 1978, 2480-82) of BnO-(CH₂)₃-OH.

$^1\text{H-NMR}$ (300 MHz, CDCl_3): δ 2.63 (dt, 2H), 3.80 (t, 2H), 4.51 (s, 2H), 7.30 (m, 5H), 9.76 (bt, 1H).

Br-(S)CH(CH₂OBn)-COOBn

(i) Br-(S)CH(CH₂OBn)-COOH

20

O-Benzylserine (3.9 g, 19 mmol) in water (10 ml) was added to a solution of sodium bromide (11 g, 107 mmol) in water (20 ml) and sulphuric acid (2 g, 20 mmol). The reaction mixture was cooled to -10°C and NaNO_2 (1.73 g, 25 mmol) was added under vigorous stirring. Another portion of water was added to the thick mixture followed, after a few minutes, by H_2SO_4 (1 g, 10 mmol). The mixture was stirred at ambient temperature over night after which it was extracted twice with EtOAc (100ml). The combined organic phase was washed twice with water and once with brine and dried (Na_2SO_4). Evaporation of the solvent gave 3.7 g (75%) of the title compound as a yellow oil which was pure enough to use directly in the next step.

35 (ii) Br-(S)CH(CH₂OBn)-COOBn

To a solution of the crude product from (i) above (2.6 g, 10

mmol) in dry benzene (25 ml) was added oxalyl chloride (2.6 g, 20.5 mmol) and molecular sieves (4 Å, 1 g). The mixture was stirred at ambient temperature under an atmosphere of Argon for 18 h. The molecular sieves was removed by 5 filtration and the solvent evaporated. The slightly yellow residue was dissolved in CH₃CN (10 ml) and benzyl alcohol (1 g, 9.2 mmol) was added. The mixture was stirred at ambient temperature for 5 h. The solvent was evaporated and the residue dissolved in Et₂O and washed once with 1 M NaOH, 10 water, brine and dried (Na₂SO₄). Evaporation of the solvent followed by flash chromatography (CH₂Cl₂/MeOH, 95/5) gave 1.8 g (67 %) of the desired compound.

15 ¹H-NMR (500 MHz, CDCl₃): δ 3.82 (dd, 1H), 3.99 (dd, 1H), 4.38 (dd, 1H), 4.56 (s, 2H), 5.23 (s, 2H), 7.23-7.46 (m, 5H).

Working Examples

Example 1

20 H-(R)Cha-Pro-Agm × 2 HOAc

(i) Boc-(R)Cha-Pro-Agm × HOAc

25 Boc-(R)Cha-Pro-OSu (1.7 mmol) and agmatine dihydrochloride (2.0 mmol, 1.18 eq) was dissolved in DMF/H₂O 95:5 (35 ml). Triethylamine was added to adjust the pH to about 10 and the solution was stirred at room temperature for 2 days. The solution was evaporated (5 mm Hg/60 °C) until dryness and 30 the crude product was purified by RPLC (CH₃CN/NH₄OAc (0.1 M), 38:62). The desired compound was obtained as a white powder after freeze-drying.

35 ¹H NMR (500 MHz, CDCl₃/DMSO-d₆ 5:2, Two rotamers, 9:1 δ (major rotamer) : 0.75-0.90 (m, 2H), 1.1-2.05 (m, 19H), 1.35 (s, 9H), 2.98-3.14 (m, 4H), 3.37 (q, 1H), 3.76 (m, 1H), 4.20 (m, 1H), 4.33 (dd, 1H), 6.30 (d, 1H), 7.05-7.80 (broad m,

5H), 8.67 (broad d, 1H).

Exchange broadened signals of the minor rotamer are unambiguously observed at δ 3.44 (m, 1H), 3.62 (m, 1H), 4.10 (m, 5 1H), 4.64 (m, 1H), 5.56 (d, 1H), 9.08 (m, 1H).

(ii) H-(R)Cha-Pro-Agm x 2 HOAc

A solution of Boc-(R)Cha-Pro-Agm (0.2 mmol) in TFA (2ml) was 10 stirred at room temperature for 4.5 h. The solvent was evaporated and the remaining oil was subjected to RPLC ($\text{CH}_3\text{CN}/\text{NH}_4\text{OAc}$ (0.1 M), 25:75). The diacetate salt was obtained as a white powder after repeated freeze-drying.

15 ^1H NMR (500.13 MHz, D_2O): δ 0.80-0.95 (m, 2H), 1.00-1.21 (m, 3H), 1.32 (m, 1H), 1.40-1.78 (m, 12H), 1.83-2.00 (m, 2H), 1.90 (s, acetate), 2.20 (m, 1H), 3.06-3.14 (m, 4H), 3.50 (m, 1H), 3.67 (m, 1H), 4.20-4.30 (m, 2H).

20 ^{13}C NMR (75.6 MHz, D_2O): guanidine: δ 157.4; carbonyl carbons: δ 169.9, 174.5.

Example 2

25 Me-(R)Cha-Pro-Agm x 2 HOAc

(i) Boc-(Me)(R)Cha-Pro-Agm

To a solution of 479.6 mg (1 mmol, 1 eq.) of 30 Boc-(Me)(R)Cha-Pro-OSu and 500 ml of NMM in 16 ml DMF/ H_2O (15/1) was added 166.5 mg (1.2 mmol, 1.2 eq.) of Agm x HCl at room temperature. The reaction was stirred an additional 70 h and the solvent was evaporated to give a crude product as an oil. This was used without purification in the next step.

35

(ii) Me-(R)Cha-Pro-Agm x 2 HOAc

The crude oil from the previous step was dissolved in 10 ml TFA/CH₂Cl₂ (1:4) at room temperature. After stirring for 2 h 25 min the solvent was evaporated and the crude product was purified with RPLC (CH₃CN/NH₄OAc(0.1M), 35/65) to give the 5 desired product as a white powder after freeze-drying.

¹H-NMR (500 MHz, D₂O): δ 0.93-1.05 (m, 2H), 1.10-1.29 (m, 3H), 1.33-1.43 (m, 1H), 1.50-1.80 (m, 12H), 1.88-2.10 (m, 2H, 10 1.92 (s, acetate), 2.27-2.36 (m, 1H), 2.68 (s, 3H), 3.15-3.23 (m, 3H), 3.24-3.31 (m, 1H), 3.57-3.66 (m, 1H), 3.76-3.83 (m, 1H), 4.28 (t, 1H), 4.39 (dd, 1H).

¹³C-NMR (125.76 MHz, D₂O): guanidine: δ 157.24; carbonyl carbons: δ 174.03, 168.24.

15

Example 3



20 (i) Boc-(R)-Cha-Pro-Agm(Z)

Boc-Agm(Z) (1 eq) was dissolved in TFA/CH₂Cl₂ (1:4, ca: 6 ml/mmol) and stirred at room temperature for ca: 2 h. The solvent was evaporated and the product dissolved together 25 with Boc-(R)Cha-Pro-OSu (1 eq) in DMF (ca: 1 ml/mmol), the pH was adjusted with NMM to ca: 9 and the mixture was stirred at room temperature for 20 h. The solvent was evaporated in vacuo, the crude product dissolved in CH₂Cl₂ and washed three times with water and once with brine. After drying (sodium 30 sulphate) the solvent was evaporated and the product flash chromatographed (CH₂Cl₂/MeOH) affording Boc-(R)Cha-Pro-Agm(Z) as a white powder.

(ii) H-(R)Cha-Pro-Agm(Z)

35

Boc-(R)Cha-Pro-Agm(Z) was dissolved in TFA/CH₂Cl₂ (1:4, ca: 6 ml/mmol) and stirred at room temperature for 2 h. The solvent

was evaporated, the product dissolved in 0.2M NaOH (20 ml/mmol) and extracted twice with dichloromethane. The organic layers were combined and washed with brine, dried (sodium sulphate) and the solvent evaporated to yield

5 H-(R)Cha-Pro-Agm(Z) as a white powder.

(iii) $\text{BnO}-(\text{CH}_2)_3-(\text{R})\text{Cha-Pro-Agm}(Z)$

H-(R)Cha-Pro-Agm(Z) (1 mmol) was dissolved in methanol (10 ml). Triethylammonium hydrochloride (1mmol), sodium cyanoborohydride (0.7 mmol) and thereafter $\text{BnO}-(\text{CH}_2)_2\text{-CHO}$ (1.05 mmol) were added and the reaction mixture stirred at room temperature over night. The solvent was evaporated and the crude product was dissolved in ethyl acetate, washed twice with water, once with brine and dried over sodium sulphate. The solvent was evaporated and the crude product was purified by flash chromatography (EtOAc/MeOH).

(iv) $\text{HO}-(\text{CH}_2)_3-(\text{R})\text{Cha-Pro-Agm} \times 2 \text{ HCl}$

20 Prepared by using deprotection procedure (d) on the product (iii) above.

25 $^1\text{H-NMR}$ (500 MHz, D_2O): δ 0.72 (m, minor rotamer), 0.84 (m, minor rotamer), 0.87-1.03 (m, 2H), 1.03-1-26 (m, 3H), 1.28-1.40 (bs, 1H), 1.44-1.80 (m, 11H), 1.80-1.95 (bs, 3H), 1.95-2.10 (bs, 2H), 2.28 (m, 1H), 3.04 (m, 1H), 3.08-3.27 (m, 5H), 3.58 (bs, 1H), 3.67 (bs, 2H), 3.78 (m, 1H), 4.12 (bd, minor rotamer), 4.30 (m,1H), 4.37 (m, 1H).

30 $^{13}\text{C-NMR}$ (125 MHz, D_2O): guanidine: δ 157.26; carbonyl carbons: δ 174.06, 168.36.

Example 4

35 $\text{HOOC-CH}_2-(\text{R})\text{Cha-Pro-Agm} \times \text{HOAc}$

General Procedure for the alkylation of the N-terminal.

This procedure is described in more general terms and will be referred to in the Examples below together with the 5 alkylating agent used in each specific Example.

The peptide to be alkylated (1 eq) and the alkylating agent (1.1-1.2 eq) were dissolved in acetonitrile (ca 10 ml/mmol). Potassium carbonate (2.0-2.2 eq) was added and the reaction 10 mixture stirred at 50-60°C until the starting material was consumed (TLC, usually 1-5 h). Filtration, evaporation of the solvent and flash chromatography ($\text{CH}_2\text{Cl}_2/\text{MeOH}$, $\text{CH}_2\text{Cl}_2/\text{MeOH}(\text{NH}_3\text{-saturated})$ or EtOAc/MeOH , ca 9/1) gave the alkylated product after evaporation of the solvent.

15

(i) $\text{BnOOC-CH}_2\text{-(R)Cha-Pro-Agm(Z)}$

Prepared from $\text{H-(R)Cha-Pro-Agm(Z)}$ (See Example 3) and $\text{Br-CH}_2\text{COOBn}$ according to the procedure described above.

20

(ii) $\text{HOOC-CH}_2\text{-(R)Cha-Pro-Agm x HOAc}$

Prepared by using the deprotection procedure (b) on the product (i) above.

25

$^1\text{H-NMR}$ (300 MHz, MeOD): δ 0.9-1.1 (m, 2H), 1.1-2.3 (m, 19H) 1.95 (s, acetate), 3.1-3.2 (m, 4H), 3.2-3.65 (m, 3H), 3.85 (m, 1H), 4.0 (bt, 1H), 4.35 (dd, 1H).

30

$^{13}\text{C-NMR}$ (75 MHz, D_2O): guanidine: δ 157.55; carbonyl carbons: δ 168.71, 171.37 and 174.3.

Example 5

35 $^i\text{Pr-OOC-CH}_2\text{-(R)Cha-Pro-Agm x HOAc}$

Alkylation as in Example 4 using $\text{H-(R)Cha-Pro-Agm(Z)}$ (See

Example 3) and Br-CH₂COOⁱPr followed by deprotection procedure (b) gave the title compound.

1H-NMR (500 MHz, MeOD): δ 0.85-1.05 (m, 2H), 1.1-1.35 (m, 9H; 5 thereof 1.23 (d, 3H), 1.25 (d, 3H)), 1.35-2.02 (m, 14H) 1.92 (s, acetate), 2.08 (m, 1H), 2.2 (m, 1H), 3.07-3.45 (m, 6H), 3.55 (m, 1H), 3.7-3.8 (m, 2H), 4.3 (dd, 1H), 5.05 (m, 1H).

13C-NMR (125 MHz, D₂O): guanidine: δ 157.39; carbonyl 10 carbons: δ 171.10, 172.76 and 174.44.

Example 6



15

(i) Me-(R)Cha-Pro-Agm(Z)

Prepared from Boc-(Me)(R)Cha-Pro-OSu in the same way as described for H-(R)Cha-Pro-Agm(Z) in Example 3.

20

(ii) HOOC-CH₂-(Me)(R)Cha-Pro-Agm × 2 TFA

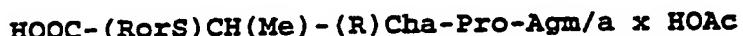
Alkylation as in Example 4 using Me-(R)Cha-Pro-Agm(Z) and Br-CH₂COOBn followed by deprotection procedure (b) gave the 25 title compound.

1H-NMR (300 MHz, D₂O): δ 0.9-1.35 (m, 6H), 1.5-2.2 (m, 14H), 2.25-2.45 (m, 1H), 3.12 (s, 3H), 3.15-3.35 (m, 4H), 3.6-3.75 (m, 1H), 3.8-3.95 (m, 1H), 4.22 (apparent bs, 2H), 4.45 (m, 30 1H), 4.6 (bt, 1H).

13C-NMR (75.47 MHz, D₂O): guanidine: δ 157.52; carbonyl carbons: δ 173.86, 168.79, 167.38.

Example 7

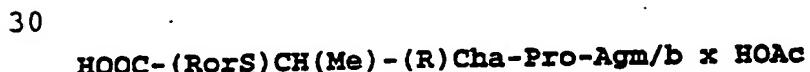
5 Alkylation as in Example 4 using H-(R)Cha-Pro-Agm(Z) (See Example 3) and Br-CH(Me)COOBn followed by deprotection procedure (a) gave the title compound as a mixture of two diastereomers.

10 Example 8

Obtained by separating the diastereomers formed in Example 7
15 using RPLC (CH₃CN/NH₄OAc (0.1M), 1/4). This diastereomer came out first of the two from the column.

1H-NMR (500 MHz, D₂O; 2 rotamers ca: 5:1 ratio): δ 0.74 (m,
minor rotamer), 1.01 (m, 2H), 1.10-1.33 (m, 3H), 1.48- 1.88
20 (m, 15H; thereof 1.51 (d, 3H)), 1.92- 2.12 (m, 3H) 1.96 (s,
acetate), 2.30 (m, 1H), 3.20 (m, 3H), 3.38 (m, 1H), 3.47 (q,
minor rotamer), 3.53-3.68 (m, 2H), 3.73 (m, 1H), 4.20 (d,
minor rotamer), 4.33 (m, 1H), 4.38 (m, 1H), 4.51 (d, minor
rotamer).

25 ¹³C-NMR (125 MHz, D₂O): guanidine: δ 157.38; carbonyl
carbons: δ 174.11, 173.45, 168.64.

Example 9

The diastereomer that came out after the first one from the column in the separation in Example 8 is the title compound
35 above.

1H-NMR (500 MHz, D₂O, 2 rotamers ca 9:1 ratio): δ 0.88 (m,

minor rotamer), 1.05 (m, 2H), 1.12-1.33 (m, 3H), 1.42 (bs, 1H), 1.50-1.88 (m, 15H; thereof 1.55 (d, 3H)), 1.93-2.13 (m, 3H) 1.95 (s, acetate), 2.30 (m, 1H), 2.40 (m, minor rotamer), 3.22 (t, 2H), 3.28 (t, 2H), 3.64 (m, 1H), 3.70 (q, 1H), 3.93 5 (t, minor rotamer), 4.35 (t, 1H), 4.41 (dd, 1H).

Example 10



10

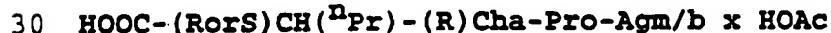
Alkylation as in Example 4 using H-(R)Cha-Pro-Agm(Z) (See Example 3) and Br-CH(ⁿPr)COOEt and deprotection procedure (e) followed by deprotection procedure (b) gave

15 HOOC-(R,S)CH(ⁿPr)-(R)Cha-Pro-Agm. The title compound was obtained by separating the diastereomers by RPLC (CH₃CN/NH₄OAc (0.1 M), 1/4) and freeze drying (H₂O) after evaporation of the solvent. This diastereomer came out first 20 of the two from the column.

20 ¹H-NMR (300 MHz, MeOD): δ 0.8-1.1 (m, 5H; thereof 0.92 (t, 3H)), 1.1-2.1 (m, 22H) 1.95 (s, acetate), 2.2 (m, 1H), 3.1-3.35 (m, 5H), 3.48 (m, 1H), 3.88 (m, 1H), 4.0 (m, 1H), 4.4 (dd, 1H).

25 ¹³C-NMR (75 MHz, D₂O): guanidine: δ 157.50; carbonyl carbons: δ 168.55 and 174.16.

Example 11



The other diastereomer from the separation in Example 10 which came out after the first one from the column is the title compound above.

35

¹H-NMR (500 MHz, MeOD): δ 0.85-1.05 (m, 5H; thereof 0.95 (t, 3H)) 1.1-2.08 (m, 22H) 1.9 (s, acetate), 2.14 (m, 1H),

3.1-3.4 (m, 5H), 3.45 (m, 1H), 3.62 (m, 1H), 3.80 (m, 1H),
4.34 (dd, 1H).

^{13}C -NMR (75 MHz, D_2O): guanidine: δ 157.53; carbonyl carbons:
5 δ 169.01 and 174.27.

Example 12

HOOC-(R or S)CH(Ph)-(R)Cha-Pro-Agm/b x HOAc

10

(i) t -BuOOCC-(R or S)CH(Ph)-(R)Cha-Pro-Agm(Z)

A mixture of H-(R)Cha-Pro-Agm(Z) (See Example 3) (0.55 mmol),
tert.butyl-(R,S)phenyl bromoacetate (0.66 mmol), K_2CO_3 (1.4
15 mmol) in CH_3CN (10 ml) was stirred at room temperature for 28 h and an additional 5 h at 60° C. The diastereomeric mixture (ca: 3:1, according to NMR) was filtered and evaporated. The remaining oil was twice subjected to flash chromatography ($\text{CH}_2\text{Cl}_2/\text{MeOH}$, 92/8), which resulted in a complete separation
20 of the two diastereomers (R_f =0.36 (minor isomer) and 0.27 (major isomer), respectively).

^1H NMR of major isomer (500.13 MHz, CDCl_3): δ 0.79 (quart, 1H), 0.90 (quart, 1H), 1.06-1.70 (m, H), 1.37 (s, 9H),
25 1.85-2.03 (m, 3H), 2.20 (m, 1H), 3.10-3.24 (m, 3H), 3.25-3.38 (m, 2H), 3.42 (m, 1H), 3.53 (m, 1H), 4.30 (s, 1H), 4.49 (dd, 1H),
5.08 (s, 2H), 7.19-7.40 (m, 10H); broad NH signals are observed in the region 6.7-8.6.

30 (ii) HOOC-(R or S)CH(Ph)-(R)Cha-Pro-Agm/b x HOAc

The major isomer (50 mmol) and thioanisole (0.5 mmol) dissolved in TFA was kept at room temperature for 8 h. After evaporation (0.1 mm Hg) for 5 h, the remaining oil was
35 purified on RPLC ($\text{CH}_3\text{CN}/\text{NH}_4\text{OAc}$ (0.1 M), 2:3) to give the title compound after evaporation of the solvent and freeze-drying.

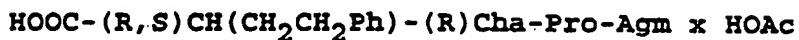
1^H NMR (500.13 MHz, MeOD): δ 0.85-1.01 (m, 2H), 1.13-1.38 (m, 4H), 1.53-2.05 (m, 14H), 1.92 (s, acetate) 2.18 (m, 1H), 3.08-3.26 (m, 3H), 3.32-3.45 (m, 2H), 3.64 (m, 1H), 3.93 (t, 1H), 4.37 (dd, 1H), 4.43 (s, 1H), 7.28-7.50 (m, 5H).

5

1³C NMR (125.6 MHz, MeOD): guanidine: δ 158.7; carbonyl carbons: δ 173.8, 174.7, 177.0.

Example 13

10



Alkylation as in Example 4 using H-(R)Cha-Pro-Agm(Z) (See Example 3) and Br-CH(CH₂-CH₂-Ph)COOEt and deprotection procedure (a) followed by deprotection procedure (e) gave HOOC-(R,S)CH(CH₂-CH₂-Ph)-(R)Cha-Pro-Agm.

Example 14

20 HOOC-(R or S)CH(CH₂CH₂Ph)-(R)Cha-Pro-Agm/a x 2 TFA

The title compound was obtained by separating the diastereomers obtained in Example 13 by RPLC (CH₃CN/NH₄OAc (0.1 M), 2/3) and freeze drying (H₂O/TFA) after evaporation of 25 the solvent. This diastereomer came out first of the two from the column is the title compound above.

1^H-NMR (500 MHz, MeOD): δ 0.93-1.11 (m, 2H), 1.24 (m, 1H), 1.29-1.40 (m, 2H), 1.52-1.85 (m, 11H), 1.89-2.11 (m, 4H), 30 2.14-2.32 (m, 3H), 2.83 (t, 2H), 3.14 (t, 2H), 3.24 (t, 2H), 3.50 (q, 1H), 3.70 (m, 1H), 4.00 (t, 1H), 4.36-4.42 (m, 2H), 7.17-7.31 (m, 5H).

1³C-NMR (125 MHz, MeOD): guanidine: δ 158.66; carbonyl 35 carbons: δ 168.08, 171.53, 174.16.

Example 15

HOOC-CH₂-CH₂-(R)Cha-Pro-Agm x HOAc(i) BnOOC-CH₂-CH₂-(R)Cha-Pro-Agm(Z)

5 Benzyl acrylate (1.1 eq) and H-(R)Cha-Pro-Agm(Z) (See Example
3) (1 eq) were dissolved in ethanol (20 ml/mmole) and stirred
at room temperature for 20 h. The solvent was evaporated and
the crude product purified by flash chromatography
(CH₂Cl₂/MeOH(NH₃-saturated), 95/5). Finally the solvent was
10 evaporated and the product dried in vacuo.

¹H-NMR (500 MHz, CDCl₃): δ 0.7-0.95 (m, 2H), 1.0-1.5 (m,
10H), 1.5-1.75 (m, 5H), 1.75-1.92 (m, 2H), 2.0 (m, 1H), 2.17
(bs, 1H), 2.45 (m, 2H), 2.63 (m, 1H), 2.79 (m, 1H), 2.97-3.25
15 (m, 4H), 3.33 (m, 2H), 3.52 (bt, 1H), 4.45 (bd, 1H),
4.95-5.12 (m, 4H), 7.13-7.4 (m, 10H).

(ii) HOOC-CH₂-CH₂-(R)Cha-Pro-Agm x HOAc

20 Prepared by using the deprotection procedure (a) on the
product (i) above.

¹H-NMR (500 MHz, D₂O): δ 0.88 (m, 2H), 1.00-1.23 (m, 3H),
1.33 (bs, 1H), 1.42-1.72 (m, 11H), 1.78-2.00 (m, 3H) 1.94
25 (s, acetate), 2.18 (m, 1H), 2.52 (m, 2H), 3.03-3.20 (m, 6H),
3.50 (m, 1H), 3.72 (m, 1H), 4.23 (m, 1H), 4.30 (m, 1H).

¹³C-NMR (125 MHz, D₂O): guanidine: δ 157.25; carbonyl
carbons: δ 178.07, 173.96, 168.24.

30

Example 16**EtOOC-CO-(R)Cha-Pro-Agm x HOAc**

35 (i) EtOOC-CO-(R)Cha-Pro-Agm(Z)

To a cold (-10° C) solution of H-(R)Cha-Pro-Agm(Z) (See

Example 3) (0.46 g, 0.89 mmol) and NMM (199 mg, 1.97 mmol) in 10 ml of THF was added Cl-COCOOEt (134 mg, 0.98 mmol) dissolved in 3 ml of THF. The mixture was kept at -10° C for one hour after which it was stirred at room temperature for 5 another hour. The solvent was evaporated and the residue was dissolved in ethyl acetate. The organic phase was washed twice with water and dried (Na_2SO_4). Evaporation of the solvent and crystallization from EtOAc gave 0.275 g (50%) of the title compound as white crystals.

10

(ii) $\text{EtOOC-CO-(R)Cha-Pro-Agm} \times \text{HOAc}$

Prepared by using the deprotection procedure (b) on the product (i) above.

15

$^1\text{H-NMR}$ (300 MHz, MeOD): δ 0.9-2.25 (m, 24H; thereof 1.17 (t, 3H)) 1.90 (s, acetate), 3.1-3.25 (m, 4H), 3.5-3.65 (m, 3H; thereof 3.59 (q, 2H)), 3.88 (m, 1H), 4.35 (m, 1H), 4.69 (dd, 1H).

20

$^{13}\text{C-NMR}$ (75.5 MHz, MeOD): guanidine: δ 157.56 and carbonyl carbons: δ 159.21, 160.74, 172.81, 174.56.

Example 17

25

 $(\text{R},\text{S})\text{Bla-(R)Cha-Pro-Agm} \times 2 \text{ TFA}$

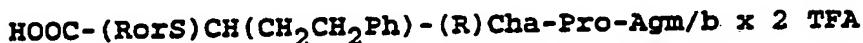
Alkylation as in Example 4 using H-(R)Cha-Pro-Agm(Z) (See Example 3) and α -bromo butyrolacton followed by deprotection 30 procedure (a) gave the title compound as a mixture of two diastereomers.

$^1\text{H-NMR}$ (500 MHz, D_2O , mixture of diastereomers ca: 1/1): δ 0.93-1.06 (m, 2H), 1.09-1.30 (m, 3H), 1.37-1.49 (m, 1H), 1.50-1.87 (m, 11H), 1.89-2.10 (m, 3H), 2.24-2.36 (m, 1H), 2.44-2.56 (m, 1H), 2.72-2.85 (m, 1H), 3.10-3.30 (m, 4H), 3.56-3.65 (m, 1H), 3.75-3.84 (m, 1H), 4.2-5.0 (m, 5H,

partially hidden by the H-O-D signal).

13C-NMR (125.76 MHz, D₂O) guanidine: δ 157.34 (peaks overlapping); carbonyl carbons: δ 174.34, 173.90, 173.62,
5 167.88, 167.58 (two peaks are overlapping).

Example 18



10

The title compound was obtained by treating the diastereomer in Example 13 by the same way as described in Example 14. This diastereomer came out after the first one from the column.

15

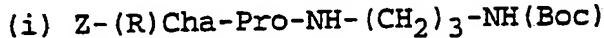
¹H-NMR (500 MHz, MeOD): δ 0.95-1.06 (m, 2H), 1.14-1.40 (m, 4H), 1.48-1.84 (m, 11H), 1.87-2.30 (m, 6H), 2.72-2.90 (m, 2H), 3.12-3.32 (m, 4H), 3.52 (m, 1H), 3.72 (m, 1H), 4.04 (dd, 1H), 4.27 (t, 1H), 4.37 (dd, 1H), 7.17-7.32 (m, 5H).

20

13C-NMR (125 MHz, MeOD): guanidine: δ 158.68; carbonyl carbons: δ 168.14, 171.46, 174.03.

Example 19

25



30 To a solution of Z-(R)Cha-Pro-OSu (1 mmol) in 1 ml of DMF at 0 °C was added H₂N-(CH₂)₃-NH(Boc) (See Preparation of starting material) dissolved in 1 ml of DMF and the pH was adjusted to ca: 9 with NMM. The reaction was stirred at room temperature for 3 days after which it was poured out on water.
35 The aqueous phase was extracted four times with EtOAc. The combined organic phase was washed twice with 0.3 M KHSO₄, 0.2 M NaOH, brine and dried. Evaporation and flash

chromatography (EtOAc/ petroleum ether, 4/1) gave the title compound in 59 % yield.

(ii) Z-(R)Cha-Pro-NH-(CH₂)₃-NH₂

5

Z-(R)Cha-Pro-NH-(CH₂)₃-NH(Boc) (0.6 mmol) was dissolved in CH₂Cl₂ (8 ml). TFA (2 ml) was added and the reaction mixture was stirred for 1 h. The solvent was evaporated and the residue was dissolved in CH₂Cl₂, washed twice with 0.2 M NaOH 10 and dried (Na₂SO₄). Evaporation of the solvent gave the amine in 93 % yield.

15 ¹H-NMR (500 MHz, CDCl₃): δ 0.79-1.03 (m, 2H), 1.05-1.75 (m, 15H), 1.84-2.08 (m, 4H), 2.36 (m, 1H), 2.66 (m, 2H), 3.25 (m, 2H), 3.43 (q, 1H), 3.85 (m, 1H), 4.45 (m, 1H), 4.56 (d, 1H) 20 5.09 (m, 2H), 5.35 (d, 1H), 7.30-7.45 (m, 5H).

(iii) Z-(R)Cha-Pro-Nag × HOAc

20 Z-(R)Cha-Pro-NH-(CH₂)₃-NH₂ (0.55 mmol, 1 eq) was dissolved in DMF (2 ml) and the pH adjusted with triethylamine to 8-9. 3,5-Dimethyl-1-pyrazolylformamidinium nitrate (0.55 mmol, 1 eq) dissolved in DMF (1 ml) was added and the reaction mixture stirred at room temperature for three days. The 25 solvent was evaporated, the crude product freeze-dried (H₂O) and purified with RPLC (CH₃CN/NH₄OAc (0.1M), 4/6) to give the title compound in 93 % yield after evaporation of the solvent and freeze-drying (H₂O).

30 (iv) H-(R)Cha-Pro-Nag × 2 HOAc

Prepared by using the deprotection procedure (a) on the product (iii) above.

35 ¹H-NMR (500 MHz, D₂O): δ 0.82-1.03 (m, 2H), 1.03-1.28 (m, 3H) 1.35 (m, 1H), 1.53-1.82 (m, 9H), 1.82-2.05 (m, 3H) 1.89 (s, acetate), 2.24 (m, 1H), 3.15 (t, 2H), 3.23 (q, 2H), 3.55 (m,

1H), 3.72 (m, 1H), 4.27-4.34 (m, 2H).

^{13}C -NMR (125 MHz, D₂O): guanidine: δ 157.37; carbonyl carbons: δ 169.81, 174.52.

5

Example 20

$n\text{Bu-}(\text{R})\text{Cha-Pro-Nag} \times 2 \text{ HOAc}$

10 (i) H-(R)Cha-Pro-Nag(Z)

Prepared from Boc-(R)Cha-Pro-OSu and Boc-Nag(Z) in the same way as described for H-(R)Cha-Pro-Agm(Z) in Example 3.

15 ^1H -NMR (500 MHz, CDCl₃): δ 0.8-1.03 (m, 2H), 1.10-1.50 (m, 6H), 1.60-1.83 (m, 8H), 1.87-2.20 (m, 3H), 3.15 (m, 1H), 3.25 (m, 2H), 3.42 (m, 2H), 3.63 (dd, 1H), 3.70 (m, 1H), 4.36 (bs, 1H), 5.07 (s, 2H), 7.22-7.43 (m, 5H).

20 (ii) $n\text{Bu-}(\text{R})\text{Cha-Pro-Nag}(Z)$

H-(R)Cha-Pro-Nag(Z) (0.5 g, 1 mmol) was dissolved in methanol (10 ml). Triethylammonium hydrochloride (0.1 g, 1mmol), sodium cyanoborohydride (44 mg, 0.7 mmol) and thereafter 25 butyric aldehyde (76 mg, 1.05 mmol) were added and the reaction mixture stirred at room temperature for 20 h. The solvent was evaporated and the crude product was dissolved in ethyl acetate, washed twice with water, once with brine and dried over sodium sulphate. The solvent was evaporated and 30 the crude product was purified by flash chromatography (EtOAc/EtOH/Et₃N, 88/10/2). Finally the solvent was evaporated and the product dried in vacuo to yield 0.22 g (40 %) of $n\text{Bu-}(\text{R})\text{Cha-Pro-Nag}(Z)$.

35 ^1H -NMR (500 MHz, CDCl₃): δ 0.82-1.0 (m, 5H; thereof 0.88 (t, 3H)), 1.08-1.49 (m, 10H), 1.58-1.8 (m, 7H), 1.88-2.22 (m, 3H), 2.4 (m, 1H), 2.5 (m, 1H), 3.05 (bs, 1H), 3.3 (m, 1H),

3.4-3.53 (m, 3H), 3.73 (m, 1H), 4.42 (bs, 1H), 5.1 (s, 2H),
7.25-7.43 (m, 5H).

(iii) $n\text{Bu}-\text{(R)Cha-Pro-Nag} \times 2 \text{ HOAC}$

5

Prepared by using the deprotection procedure (a) on the product (ii) above.

10 $^1\text{H-NMR}$ (300 MHz, D_2O): δ 0.94 (t, 2H), 1.10-1.31 (m, 3H),
1.38 (m, 3H), 1.55-1.88 (m, 11H), 1.88-2.15 (m, 3H) 1.95 (s,
acetate), 2.34 (m, 1H), 2.95 (m, 1H), 3.08 (m, 1H), 3.24 (t,
2H), 3.30 (m, 2H), 3.66 (m, 1H), 3.82 (m, 1H), 4.32 (t, 1H),
4.41 (dd, 1H).

15 $^{13}\text{C-NMR}$ (125 MHz, D_2O): guanidine: δ 157.40; carbonyl
carbons: δ 180.39, 174.28, 168.55.

Example 21

20 $\text{HO-(CH}_2)_3-\text{(R)Cha-Pro-Nag} \times 2 \text{ TFA}$

(i) $\text{BnO-(CH}_2)_3-\text{(R)Cha-Pro-Nag(Z)}$

25 1-Benzylxy 3-trifluoromethanesulfonylpropane (See Prep. of
Starting Materials) (0.5 g, 1 mmol) and $\text{H-(R)Cha-Pro-Nag(Z)}$
(See Example 20) were dissolved in tetrahydrofuran (10 ml).
Potassium carbonate (0.28 g, 2 mmol) was added and the
reaction mixture was stirred at room temperature for two
hours. The solvent was evaporated and the crude product
30 extracted with ethyl acetate/water. The organic phase was
washed once with aqueous sodium hydrogen carbonate, once with
water and once with brine. After drying over sodium sulphate
the solvent was evaporated and the crude product flash
chromatographed ($\text{CH}_2\text{CH}_2/\text{MeOH(NH}_3\text{-saturated})$, 95:5). Finally
35 the solvent was evaporated and the product dried in vacuo to
yield 0.29 g (45%) of the title compound.

¹H-NMR (500 MHz, CDCl₃): δ 0.77-1.03 (m, 2H), 1.03-2.18 (m, 19H), 2.52 (m, 1H), 2.64 (m, 1H), 3.03 (bs, 1H), 3.1-3.6 (m, 7H), 3.66 (m, 1H), 4.41 (bs, 1H), 4.46 (s, 2H), 5.08 (s, 2H), 7.2-7.4 (m, 5H), 7.55 (m, 1H).

5

(ii) HO-(CH₂)₃-(R)Cha-Pro-Nag × 2 TFA

Prepared by using the deprotection procedure (a) on the product (i) above.

10

¹H-NMR (500 MHz, D₂O): δ 1.00 (bs, 2H), 1.10-1.32 (m, 3H), 1.40 (bs, 1H), 1.55-2.15 (m, 14H), 2.30 (m, 1H), 3.05-3.35 (m, 6H), 3.57-3.75 (m, 3H), 3.81 (bs, 1H), 4.35 (bs, 1H), 4.42 (bs, 1H).

15

Example 22

HOOC-CH₂-(R)Cha-Pro-Nag × HOAc

20 (i) H-(R)Cha-Pro-NH-(CH₂)₃-N₃

Prepared in the same way as H-(R)Cha-Pro-Agm(Z) (See Example 3) starting from Boc-(R)Cha-Pro-OSu and Boc-NH-(CH₂)₃-N₃ (replacing Boc-Agm(Z)).

25

(ii) EtOOC-CH₂-(R)Cha-Pro-NH-(CH₂)₃-NH₂ × HOAc

Alkylation as in Example 4 using H-(R)Cha-Pro-NH-(CH₂)₃-N₃ and EtOOC-CH₂-Br followed by deprotection procedure (a) to 30 reduce the azide gave the title compound.

(iii) EtOOC-CH₂-(R)Cha-Pro-Nag × HOAc

The same procedure as described in Example 19 (iii) for 35 Z-(R)Cha-Pro-Nag was used to accomplish the guanidation of the amine from (ii) above. The title compound was obtained in a pure form after RPLC (CH₃CN/NH₄OAc (0.1M), 3/7) evaporation

of the solvent and freeze drying (H_2O).

(iv) $\text{HOOC-CH}_2-(\text{R})\text{Cha-Pro-Nag} \times \text{HOAc}$

5 Prepared by using the deprotection procedure (e) on the
product (iii) above.

10 $^1\text{H-NMR}$ (500 MHz, D_2O): δ 0.99 (m, 2H), 1.09-1.30 (m, 3H),
 1.44 (m, 1H), 1.59-2.09 (m, 12H) 1.92 (s, acetate), 2.29 (m,
1H), 3.20 (t, 2H), 3.28 (m, 2H), 3.52-3.63 (m, 3H), 3.76 (m,
1H), 4.38 (dd, 1H), 4.42 (t, 1H).

15 $^{13}\text{C-NMR}$ (125 MHz, D_2O): guanidine: δ 157.43; carbonyl
carbons: δ 168.72, 171.36, 174.35.

15

Example 23

$\text{EtOOCC-CH}_2-(\text{R})\text{Cha-Pro-Nag} \times \text{HOAc}$

20 Prepared according to example 22 (iii).

10 $^1\text{H-NMR}$ (300 MHz, D_2O): δ 1.07 (m, 2H), 1.17-1.59 (m, 7H;
thereof 1.38 (t, 3H)), 1.60-2.24 (m, 12H) 2.04 (s, acetate),
2.39 (m, 1H), 3.31 (t, 2H), 3.39 (t, 2H), 3.63-3.90 (m, 4H),
4.12 (t, 1H), 4.36 (q, 2H), 4.46 (dd, 1H).

15 $^{13}\text{C-NMR}$ (75 MHz, D_2O): guanidine: δ 157.37; carbonyl
carbons: δ 173.73, 175.09, 175.70.

30 Example 24

$i\text{PrOOCC-CH}_2-(\text{R})\text{Cha-Pro-Nag} \times \text{HOAc}$

Alkylation as in Example 4 using H-(R)Cha-Pro-Nag(Z) (See
35 Example 20) and $\text{Br-CH}_2\text{COO}^i\text{Pr}$ followed by deprotection
procedure (b) gave the title compound.

¹H-NMR (500 MHz, MeOD): δ 0.85-1.05 (m, 2H), 1.1-2.15 (m, 22H; thereof 1.23 (d, 3H), 1.25 (d, 3H)), 1.92 (s, acetate), 2.2 (m, 1H), 3.10-3.35 (m, 5H), 3.4 (m, 1H), 3.55 (m, 1H), 3.65-3.8 (m, 2H), 4.28 (dd, 1H), 5.03 (m, 1H).

5

¹³C-NMR (125 MHz, D₂O): guanidine: δ 157.39; carbonyl carbons: δ 170.40, 172.00 and 174.50.

Example 25

10

t_{Bu}OOC-CH₂-(R)Cha-Pro-Nag x 2 TFA

Alkylation as in Example 4 using H-(R)Cha-Pro-Nag(Z) (See Example 20) and Br-CH₂COOt_{Bu} followed by deprotection 15 procedure (b) gave the title compound.

¹H-NMR (300 MHz, MeOD): δ 0.9-1.15 (m, 2H), 1.15-2.15 (m, 25H; thereof 1.55 (bs, 9H)), 2.3 (m, 1H), 3.15-3.45 (m, 4H), 3.55 (m, 1H), 3.7-3.95 (m, 3H), 4.3-4.4 (m, 2H).

20

¹³C-NMR (75 MHz, D₂O): guanidine: δ 157.55; carbonyl carbons: δ 166.55, 168.13 and 174.33.

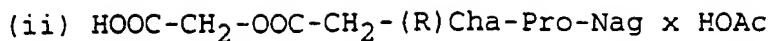
Example 26

25

HOOC-CH₂-OOC-CH₂-(R)Cha-Pro-Nag x HOAc

(i) BnOOC-CH₂-OOC-CH₂-(R)Cha-Pro-Nag(Z)

30 H-(R)Cha-Pro-Nag(Z) (See Example 20), 0.20 g (0.40 mmol), was mixed with 0.115 g (0.40 mmol) of benzyloxycarbonylmethyl bromoacetate, 55 mg of K₂CO₃ (0.40 mmol) and 5 ml of CH₃CN. The mixture was stirred at room temperature for 6 h. The solvent was evaporated and the crude product chromatographed 35 (CH₂Cl₂/MeOH, 9/1) to give 0.20 g (71%) of the desired compound after evaporation of the solvent.



Prepared by using the deprotection procedure (a) on the product (i) above.

5

¹H-NMR (500 MHz, MeOD): δ 0.85-1.1 (m, 2H), 1.1-1.6 (m, 8H), 1.6-2.15 (m, 10H) 1.99 (s, acetate), 2.23 (m, 1H), 3.1-3.4 (m, 4H), 3.45-3.65 (m, 4H), 3.7-3.9 (m, 3H), 4.34 (m, 1H), 4.48 (dd, 2H).

10

¹³C-NMR (125 MHz, MeOD), guanidine: δ 158.8; carbonyl carbons: δ 176.1, 175.2, 174.9, 173.1.

Example 27

15



Alkylation as in Example 4 using H-(R)Cha-Pro-Nag(Z) (See Example 20) and Cl-CH₂CONH₂, in the presence of a catalytic (10 mol%) amount of KI in the reaction, followed by deprotection procedure (a) gave the title compound.

¹H-NMR (500 MHz, D₂O): δ 1.02 (m, 2H), 1.12-1.34 (m, 3H), 1.46 (m, 1H), 1.61-2.13 (m, 9H) 1.99 (s, acetate), 2.34 (m, 1H), 3.25 (t, 2H), 3.33 (t, 2H), 3.60-3.82 (m, 4H), 4.22 (t, 1H), 4.41 (dd, 1H).

¹³C-NMR (75 MHz, D₂O): guanidine: δ 157.5; carbonyl carbons: δ 168.94, 169.40, 174.43.

30

Example 28



35 Alkylation as in Example 4 using H-(R)Cha-Pro-Nag(Z) (See Example 20) and Br-CH₂CONHCH₂COOBn (See Prep. of starting materials) followed by deprotection procedure (a) gave the

title compound.

1^H-NMR (500 MHz, MeOD): δ 1.01 (m, 2H), 1.15-1.38 (m, 3H), 1.47 (m, 1H), 1.64-2.13 (m, 12H), 2.27 (m, 1H), 3.17-3.26 (m, 5 3H), 3.37 (m, 1H), 3.51 (m, 1H), 3.83 (m, 1H), 3.88 (s, 2H), 3.93-4.06 (m, 2H), 4.35-4.45 (m, 2H).

13^C-NMR (75 MHz, MeOD): guanidine: δ 158.71; carbonyl carbons: δ 166.94, 168.35, 172.44, 174.17.

10

Example 29

15

(HOOC-CH₂)₂-(R)Cha-Pro-Nag x HOAc

(i) (EtOOC-CH₂)₂-(R)Cha-Pro-NH-(CH₂)₃-NH₂ x HOAc

20 Alkylation as in Example 4 using H-(R)Cha-Pro-NH-(CH₂)₃-N₃ (See Example 22) and Br-CH₂COOEt (10 eq. was used to accomplish the dialkylation) followed by deprotection procedure (a) gave the title compound.

25 (ii) (EtOOC-CH₂)₂-(R)Cha-Pro-Nag x HOAc

The same procedure as described in Example 19 (iii) for Z-(R)Cha-Pro-Nag was used to accomplish the guanidation of the amine above. Purification of the compound was made with 30 RPLC (CH₃CN/NH₄OAc (0.1M), 4:6)

(iii) (HOOC-CH₂)₂-(R)Cha-Pro-Nag x HOAc

35 The hydrolysis of the ester groups was made according to deprotection procedure (e) using a double amount of NaOH. The final compound was obtained pure after RPLC (CH₃CN/NH₄OAc (0.1M), 2:8), evaporation of the solvent and freeze drying

(H₂O).

¹H-NMR (300 MHz, D₂O): δ 0.92-1.49 (m, 6H), 1.60-2.54 (m, 10H) 2.05 (s, acetate), 3.25-3.50 (m, 4H), 3.65-4.03 (m, 6H; thereof 3.95 (s, 4H)), 4.49 (m, 1H), 4.71 (m, 1H; partly hidden by the H-O-D peak).

¹³C-NMR (75 MHz, D₂O): guanidine: δ 157.64; carbonyl carbons: δ 168.62, 171.39, 174.30.

10

Example 30

15



(i) Me-(R)Cha-Pro-Nag(Z)

20 Prepared from Boc-(Me)(R)Cha-Pro-OSu and Boc-Nag(Z) in the same way as described for H-(R)Cha-Pro-Agm(Z) in Example 3.

(ii) HOOC-CH₂-(Me)(R)Cha-Pro-Nag × 2 TFA

25 Alkylation as in Example 4 using Me-(R)Cha-Pro-Nag(Z) and Br-CH₂COOBn followed by deprotection procedure (b) gave the title compound.

¹H-NMR (500 MHz, D₂O): δ 0.8-1.06 (m, 2H), 1.08-1.27 (m, 4H), 1.55-2.10 (m, 12H), 2.30 (m, 1H); 3.04 (s, 3H), 3.14-3.33 (m, 4H), 3.63 (m, 1H), 3.81 (m, 1H), 4.13 (apparent bs, 2H), 4.38 (br.dd, 1H), 4.56 (bt, 1H).

¹³C-NMR (125.76 MHz, D₂O): guanidine: δ 157.40; carbonyl carbons: δ 174.05, 168.83, 167.44.

Example 31

HOOC-CH₂-(ⁿBu)(R)Cha-Pro-Nag x 2 TFA

Alkylation as in Example 4 using ⁿBu-(R)Cha-Pro-Nag(Z) (See Example 20) and Br-CH₂COOBn followed by deprotection
5 procedure (a) gave the title compound.

¹H-NMR (500 MHz, D₂O): δ 0.78-0.88 (m, 3H), 0.88-1.02 (m, 2H), 1.02-1.23 (m, 4H), 1.23-1.38 (m, 2H), 1.45-1.84 (m, 11H), 1.84-2.10 (m, 3H), 2.24 (m, 1H), 3.05-3.18 (m, 3H),
10 3.18-3.38 (m, 3H), 3.57 (m, 1H), 3.77 (m, 1H), 4.05-4.25 (m, 2H), 4.32 (m, 1H), 4.50 (m, 1H).

¹³C-NMR (125 MHz, D₂O): guanidine: δ 159.17; carbonyl carbons: δ 175.66, 171.13, 169.31.

15

Example 32**HOOC-(R,S)CH(Me)-(R)Cha-Pro-Nag x HOAc**

20 Alkylation as in Example 4 using H-(R)Cha-Pro-Nag(Z) (See Example 20) and Br-CH(Me)COOBn followed by deprotection procedure (a) gave the title compound as a mixture of two diastereomers.

25 **Example 33****HOOC-(R or S)CH(Me)-(R)Cha-Pro-Nag/a x HOAc**

Obtained by separating the diastereomers formed in Example 32
30 using RPLC (CH₃CN/NH₄OAc (0.1M), 1/4) followed by evaporation of the solvent. This diastereomer came out first of the two from the column.

¹H-NMR (300 MHz, D₂O, 2 rotamers ca: 9:1 ratio): δ 0.78 (m, minor rotamer), 1.07 (m, 2H), 1.17-1.42 (m, 3H), 1.48-1.64 (m, 4H; thereof 1.56 (d, 3H)), 1.64-1.95 (m, 9H), 1.95-2.20 (m, 3H) 2.00 (s, acetate), 2.37 (m, 1H), 3.28 (t, 2H), 3.38

(t, 2H), 3.53 (m, minor rotamer), 3.63 (m, 2H), 3.77 (m, 1H), 4.24 (d, minor rotamer), 4.35-4.50 (m, 2H), 4.60 (d, minor rotamer).

5 Example 34



The title compound was obtained by using the same procedure
10 as described in Example 33 on the compound formed in Example
32. This diastereomer came out after the first one from the
column.

15 $^1\text{H-NMR}$ (300 MHz, D_2O , 2 rotamers ca: 9:1 ratio): δ 0.95 (m,
minor rotamer), 1.12 (m, 2H), 1.22-1.40 (m, 3H), 1.40-1.67
(m, 4H; thereof 1.60 (d, 3H)), 1.67-2.00 (m, 9H), 2.00-2.25
(m, 3H) 2.03 (s, acetate), 2.40 (m, 1H), 3.25-3.48 (m, 4H),
3.66-3.84 (m, 2H), 3.93 (m, 1H), 4.38 (m, 1H), 4.50 (m, 1H),
4.93 (m, minor rotamer).

20 20 $^{13}\text{C-NMR}$ (75.5 MHz, D_2O): δ 157.42; carbonyl carbons: δ
168.05, 171.99, 174.04.

Example 35

25 Etooc-(R,S)CH(Me)-(R)Cha-Pro-Nag x 2 TFA

Prepared in the same way as described for Example 22 using
EtoOOC-CH(Me)-Br instead of Br-CH₂-COOEt in the alkylation.

30 30 $^1\text{H-NMR}$ (500 MHz, MeOD, 2 diastereomers ca: 2.5:1 ratio and 4
rotamers): δ 0.88-2.43 (m, 25H), 3.1-4.55 (m, 11H).

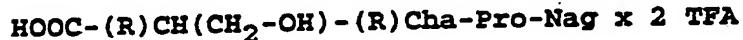
35 35 $^{13}\text{C-NMR}$ (75 MHz, MeOD): guanidine: δ 158.65; carbonyl
carbons: δ 174.33, 170.66, 168.20.

Example 36

5 Alkylation as in Example 4 using H-(R)Cha-Pro-Nag(Z) (See Example 20) and Br-CH(ⁿPr)COOEt and deprotection procedure (e) followed by deprotection procedure (b) gave
HOOC-(R,S)CH(ⁿPr)-(R)Cha-Pro-Agm. The title compound was obtained by separating the diastereomers (this diastereomer
10 came out first of the two from the column) by RPLC
(CH₃CN/NH₄OAc (0.1 M), 1/4) and freeze drying (H₂O) after evaporation of the solvent.

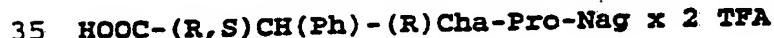
15 ¹H-NMR (500 MHz, MeOD): δ 0.85 -1.05 (m, 5H; thereof 0.95 (t, 3H)), 1.1-2.05 (m, 20H), 1.95 (s, acetate), 2.18 (m, 1H), 3.15-3.3 (m, 4H), 3.35 (m, 1H), 3.46 (m, 1H), 3.85 (m, 1H), 4.04 (m, 1H), 4.38 (dd, 1H).

20 ¹³C-NMR (125 MHz, MeOD): guanidine: δ 158.73; carbonyl carbons: δ 171.63, 174.43 and 176.78.

Example 37

25 Alkylation as in Example 4 using H-(R)Cha-Pro-Nag(Z) (See Example 20) and Br-(S)CH(CH₂-OBn)-COOBn followed by deprotection procedure (a) gave the title compound.

30 ¹H-NMR (300 MHz, D₂O): δ 0.75-1.56 (m, 7H), 1.56-2.30 (m, 11H), 2.40 (m, 1H), 3.15-3.55 (m, 4H), 3.55-4.60 (m, 7H).

Example 38

Alkylation as in Example 4 using H-(R)Cha-Pro-Nag(Z) (See

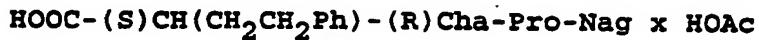
Example 20) and Br-CH(Ph)COO^tBu and deprotection procedure (a) followed by (f) gave the title compound as a mixture of two diastereomers.

5 ¹H-NMR (300 MHz, MeOD): δ 0.8-1.1 (m, 2H), 1.1-2.18 (m, 16H), 2.26 (m, 1H), 3.04-3.35 (m, 5H), 3.45 (m, 1H), 3.7 (m, 1H), 4.35 (m, 1H), 4.85 (s, 1H, one isomer), 5.05 (s, 1H, the other isomer), 7.4-7.6 (m, 5H), 7.75 (bt, 1H).

10 ¹³C-NMR (75 MHz, D₂O): guanidine: δ 158.68; carbonyl carbons: δ 174.39, 174.15 and 170.5, 170.06 and 168.32, 167.78.

Example 39

15

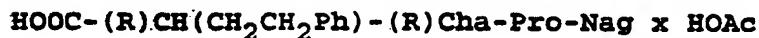


Alkylation as in Example 21 using H-(R)Cha-Pro-Nag(Z) (See Example 20) and TfO-(R)CH(CH₂CH₂Ph)-COOEt and deprotection 20 procedure (e) followed by (a) gave the title compound.

¹H-NMR (300 MHz, MeOD): δ 0.77-1.05 (m, 2H), 1.05-1.35 (m, 5H), 1.35-2.16 (m, 14H) 1.88 (s, acetate), 2.71 (t, 2H), 3.07-3.53 (m, 7H), 3.73 (m, 1H), 4.32 (m, 1H), 7.03-7.25 (m, 25 5H).

¹³C-NMR (75 MHz, MeOD): guanidine: δ 158.71; carbonyl carbons: δ 174.15, 177.31, 182.61.

30 Example 40



Alkylation as in Example 4 using H-(R)Cha-Pro-Nag(Z) (See 35 Example 20) and Br-CH(CH₂CH₂Ph)COOEt followed by deprotection procedure (a) and (e) gave HOOC-(R,S)CH(CH₂-CH₂-Ph)-(R)Cha-Pro-Nag. The title compound was obtained by separating

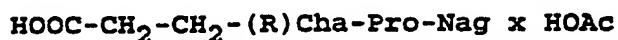
the two diastereomers with RPLC ($\text{CH}_3\text{CN}/\text{NH}_4\text{OAc}$ (0.1 M), 2/3) and freeze drying (H_2O) after evaporation of the solvent.

$^1\text{H-NMR}$ (300 MHz, MeOD): δ 0.97 (m, 2H), 1.10-1.41 (m, 3H),
5 1.43-2.30 (m, 16H) 1.96 (s, acetate), 2.70 (m, 2H), 3.06-3.26
(m, 3H), 3.28-3.66 (m, 3H), 3.84 (m, 1H), 4.14 (bt, 1H), 4.39
(dd, 1H), 7.11-7.28 (m, 5H).

$^{13}\text{C-NMR}$ (75 MHz, MeOD): guanidine: δ 158.66

10

Example 41



15 (i) EtOOC-CH2-CH2-(R)Cha-Pro-NH-(CH2)3-NH2

Alkylation as described in Example 15 using
H-(R)Cha-Pro-NH-(CH₂)₃-N₃ instead of H-(R)Cha-Pro-Agm(Z)
followed by deprotection procedure (a) gave the title
20 compound.

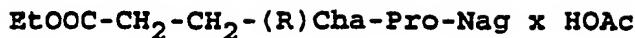
(ii) Et-OOC-CH2-CH2-(R)Cha-Pro-Nag x HOAc

Guanidation of the amine above in the same way as described
25 in Example 19 for Z-(R)Cha-Pro-Nag gave the title compound
(ii).

(iii) HOOC-CH2-CH2-(R)Cha-Pro-Nag x HOAc

30 Prepared by using the deprotection procedure (e) on the
product (ii) above.

$^1\text{H-NMR}$ (500 MHz, D₂O): δ 1.12 (m, 2H), 1.22-1.48 (m, 3H), 1.54
(bs, 1H), 1.70-2.37 (m, 12H) 2.14 (s, acetate), 2.53 (m,
35 1H), 2.70 (bs, 2H), 3.15 (t, 1H), 3.25-3.55 (m, 5H), 3.75 (m,
1H), 3.93 (m, 1H), 4.43 (t, 1H), 4.52 (m, 1H).

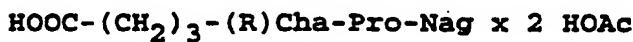
Example 42

5 Prepared according to Example 41 (ii).

¹H-NMR (500 MHz, D₂O): δ 0.97 (m, 2H), 1.11-1.39 (m, 7H; thereof 1.30 (t, 3H)), 1.50 (t, 2H), 1.62-1.76 (m, 5H), 1.76-2.14 (m, 5H) 1.93 (s, acetate), 2.29 (m, 1H), 2.62 (t, 2H), 2.77-2.94 (m, 2H), 3.23 (t, 2H), 3.32 (t, 2H), 3.60-3.87 (m, 3H), 4.20 (q, 2H), 4.36 (dd, 1H).

¹³C-NMR (125 MHz, D₂O): guanidine: δ 157.39; carbonyl carbons: δ 182.05, 175.13, 175.02.

15

Example 43

20 (i) Et-OOC-CH=CH-CH₂-(R)Cha-Pro-Nag(Z)

H-(R)Cha-Pro-Nag(Z) (See Example 20) (1 eq) and ethyl 3-bromocrotonate (1.1 eq) were dissolved in acetonitrile (15 ml/mmole). Potassium carbonate was added and the reaction mixture stirred at room temperature for 2 h. After filtration and evaporation of the solvent, the crude product was purified by flash chromatography (CH₂Cl₂/MeOH). Finally the solvent was evaporated and product dried in vacuo.

30 ¹H-NMR (500 MHz, CDCl₃): δ 0.73-1.0 (m, 2H), 1.0-1.4 (m, 8H; thereof 1.33 (t, 3H)), 1.43-2.15 (m, 12H), 2.96 (bs, 1H), 3.12 (dd, 1H), 3.16-3.48 (m, 6H), 3.56 (m, 1H), 4.15 (q, 2H), 4.35 (bs, 1H), 5.03 (s, 1H), 6.0 (d, 1H), 6.85 (dt, 1H), 7.05 (bs, 1H), 7.17-7.37 (m, 5H), 7.5 (bs, 1H).

35

(ii) EtOOC-(CH₂)₃-(R)Cha-Pro-Nag × 2 TFA

Prepared by using the deprotection procedure (a) on the product (i) above.

(iii) HOOC-(CH₂)₃-(R)Cha-Pro-Nag × 2 HOAc

5

Prepared by using the deprotection procedure (e) on the product (ii) above.

10 ¹H-NMR (500 MHz, D₂O): δ 1.02 (bs, 2H), 1.08-1.32 (m, 3H),
1.42 (bs, 1H), 1.55-2.15 (m, 14H) 1.92 (s, acetate), 2.33
(bs, 3H), 3.00 (bs, 1H), 3.07 (bs, 1H), 3.18-3.40 (m, 4H),
3.62 (bs, 1H), 3.82 (bs, 1H), 4.33 (bs, 1H), 4.40 (bs, 1H).

15 ¹³C-NMR (125 MHz, D₂O): guanidine: δ 157.42; carbonyl
carbons: δ 181.87, 174.34, 168.64.

Example 44

EtOOC-(CH₂)₃-(R)Cha-Pro-Nag × 2 TFA

20

Prepared according to Example 43 (ii).

25 ¹H-NMR (300 MHz, MeOD/D₂O): δ 0.63-1.30 (m, 9H; thereof 1.02
(t, 3H)), 1.30-1.97 (m, 14H), 2.06 (bs, 1H), 2.28 (m, 2H),
2.72-3.20 (m, 6H), 3.36 (m, 1H), 3.60 (m, 1H), 3.94 (m, 2H),
4.06 (m, 1H), 4.17 (m, 1H).

30 ¹³C-NMR (75 MHz, MeOD/D₂O): guanidine: δ 158.10; carbonyl
carbons: δ 175.40, 174.23, 168.54.

30

Example 45

HOOC-CO-(R)Cha-Pro-Nag × HOAc

35 (i) EtOOC-CO-(R)Cha-Pro-Nag(Z)

H-(R)Cha-Pro-Nag(Z), 0.50 g (0.97 mmol) was dissolved in 0.54

ml triethyl amine and 8 ml of CH_2Cl_2 . Ethyl oxalylchloride, 0.146 g (1.07 mmol) dissolved in 2 ml of CH_2Cl_2 was added while the temperature rose from 22-28°C and the reaction was stirred at room temperature for 2 h. The organic phase was 5 washed twice with water, dried (Na_2SO_4) and flash chromatographed (EtOAc/EtOH(99%), 9/1) to give 92 mg (15 %) of the title compound.

(ii) HOOC-CO-(R)Cha-Pro-Nag x HOAc

10

Using the deprotection procedure (b) followed by (e) gave the title compound.

$^1\text{H-NMR}$ (300 MHz, MeOD): δ 0.88-1.14 (m, 2H), 1.15-1.5 (m, 15 4H), 1.5-2.3 (m, 13H) 1.9 (s, acetate), 3.1-3.43 (m, 4H), 3.6 (m 1H), 4.05 (m, 1H), 4.43 (dd, 1H), 4.5 (m, 1H).

$^{13}\text{C-NMR}$ (75 MHz, D_2O): guanidine: δ 157.57; carbonyl carbons: δ 165.94, 173.95, 174.85 and 181.22.

20

Example 46

MeOOC-CO-(R)Cha-Pro-Nag x HOAc

25 (i) MeOOC-CO-(R)Cha-Pro-Nag(Z)

The methyl ester was obtained by transesterification of EtOOC-CO-(R)Cha-Pro-Nag(Z) (See Example 45) on the column during flash chromatography when EtOAc/MeOH(9:1) was used as 30 eluent. Yield 55%.

(ii) MeOOC-CO-(R)Cha-Pro-Nag x HOAc

Prepared by using the deprotection procedure (b) on the 35 product (i) above.

$^1\text{H-NMR}$ (300 MHz, MeOD): δ 0.9-1.1 (m, 2H), 1.1-2.3 (m, 17H)

1.9 (s, acetate), 3.12-3.4 (m, 4H), 3.52-3.67 (m, 2H), 3.9 (s, 3H), 4.35 (m, 1H), 4.65 (m, 1H).

13C-NMR (75MHz, D₂O): guanidine: δ 157.52; carbonyl carbons: 5 δ 159.11, 161.20 173.17 and 174.90.

Example 47

(R,S)Bla-(R)Cha-Pro-Nag × 2 TFA

10

Alkylation as in Example 4 using H-(R)Cha-Pro-Nag(Z) (See Example 20) and α-bromo butyrolacton followed by deprotection procedure (a) gave the title compound as a mixture of two diastereomers.

15

1H-NMR (300 MHz, D₂O, mixture of diastereomers): δ 1.0-1.43 (m, 5H), 1.45-1.60 (br.s, 1H), 1.64-2.28 (m, 12H), 2.31-2.50 (m, 1H), 2.80-2.98 (m, 1H), 3.23-3.46 (m, 4H), 3.66-3.79 (m, 1H), 3.82-3.96 (m, 1H), 4.33-5.08 (m, 5H, partially hidden by 20 the H-O-D signal).

Example 48

HOOC-(R,S)CH(CH₂COOH)-(R)Cha-Pro-Nag × HOAc

25

(i) BnOOC-(R,S)CH(CH₂COOBn)-(R)Cha-Pro-Nag(Z)

H-(R)Cha-Pro-Nag(Z) (See Example 20), 0.21 g (0.42 mmol), and 0.12 g (0.42 mmol) of dibenzyl maleate were dissolved in 10 30 ml of CH₃CN. The mixture was refluxed over night, evaporated and flash chromatographed (CH₂Cl₂/MeOH, 94/6). Evaporation of the solvent gave the desired compound in 22 % yield.

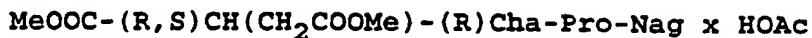
(ii) HOOC-(R,S)CH(CH₂COOH)-(R)Cha-Pro-Nag × HOAc

35

Prepared by using the deprotection procedure (a) on the product (i) above.

¹H-NMR (500 MHz, MeOD): δ 0.9-2.4 (m, 19H), 2.00 (s, acetate) 2.7-3.0 (m, 2H), 3.1-3.6 (m, 5H), 3.75-3.9 (m, 2H), 4.2-4.5 (m, 2H).

5 Example 49



(i) MeOOC-(R,S)CH(CH₂COOMe)-(R)Cha-Pro-Nag (Z)

10 H-(R)Cha-Pro-Nag (Z) (See Example 20), 0.21 g (0.42 mmol), and 0.24 g (1.7 mmol) of dimethyl maleate were dissolved in 15 ml of MeOH. The mixture was refluxed over night, evaporated and flash chromatographed (CH₂Cl₂/MeOH, 9/1). Evaporation of the 15 solvent gave the desired compound in 45% yield.

(ii) MeOOC-(R,S)CH(CH₂COOMe)-(R)Cha-Pro-Nag x HOAc

Prepared by using the deprotection procedure (c) on the 20 product (i) above.

¹H-NMR (500 MHz, MeOD): δ 0.85-1.1 (m, 2H), 1.15-2.3 (m, 17H), 1.91 (s, acetate), 2.6-2.8 (m, 2H), 3.1-3.5 (m, 5H), 3.5-3.8 (m, 10H; thereof 4 singlets 3.66, 3.68, 3.71, 3.73), 25 4.29 (m, 1H).

Example 50



30

(i) ^tBuOOC-Ph-4-CH₂-(R)Cha-Pro-NH-(CH₂)₃-N₃

H-(R)Cha-Pro-NH-(CH₂)₃-N₃ (See Example 22), 0.39 g (1.1 mmol) and 0.33 g (1.2 mmol) of tertiarybutyl p-bromomethylbenzoate 35 were dissolved in 10 ml of CH₃CN and 0.19 g (2.4 mmol) of K₂CO₃ was added. The mixture was refluxed over night and evaporated. The crude product was flash chromatographed

(CH₂Cl₂/MeOH, 92:8) to give 0.50 g (84%) of the title compound.

(ii) ^tBuOOC-Ph-4-CH₂-(R)Cha-Pro-NH-(CH₂)₃-NH₂

5

To a solution of 0.60 g (1.8 mmol) of bis-phenylthio stannane, 0.20 g (1.8 mmol) of thiophenol and 0.18 g (1.8 mmol) of triethyl amine in 50 ml of CH₂Cl₂ at 0°C was added 0.50 g (0.92 mmol) of

10 ^tBuOOC-Ph-4-CH₂-(R)Cha-Pro-NH-(CH₂)₃-N₃. The mixture was stirred at 0°C for 30 min. and at room temperature for 4 h. It was then diluted with CH₂Cl₂ and washed with aqueous sodium bicarbonate and subsequently 3 times with 2% H₂O₂. The organic layer was extracted with dilute HCl. The combined 15 acidic water phase was washed with EtOAc and subsequently made alkaline with NaOH(aq). The aqueous layer was extracted twice with ethyl acetate. The combined organic layer was dried (Na₂SO₄) and evaporated. Flash chromatography (CH₂Cl₂/MeOH(NH₃-saturated), 8:2) gave 0.12g (26%) of the 20 title compound.

(iii) HOOC-Ph-4-CH₂-(R)Cha-Pro-Nag x 2 TFA

Guanidation of the amine above in the same way as described 25 in Example 19 for Z-(R)Cha-Pro-Nag followed by deprotection procedure (f) gave the title compound.

¹H-NMR (500 MHz, MeOD): δ 0.9-1.5 (m, 7H), 1.4-1.9 (m, 9H), 1.95-2.1 (m, 2H), 2.16 (m, 1H), 2.32 (m, 1H), 3.2-3.3 (m, 30 3H), 3.41 (pentet, 1H), 3.53 (m, 1H), 3.77 (m, 1H), 4.2-4.3 (m, 3H), 4.42 (dd, 1H), 7.15 (d, 2H), 8.10 (d, 2H).

¹³C-NMR (125 MHz, MeOD), guanidine: δ 160.8; carbonyl carbons: δ 174.3, 168.9, 168.2.

Example 51

5 ($\text{EtO})_2\text{PO-CH}_2-(\text{R})\text{Cha-Pro-Nag(Z)}$ (See Example 53), 60 mg (92 mmol), was dissolved in 3 ml of CH_3CN . Trimethylsilyl bromide, 0.15 ml, was added and the mixture was left at room temperature for 21 h. After evaporation and NMR analysis it was found that some ester remained. The crude material was
10 again dissolved in 3 ml of CH_3CN and 0.15 ml of trimethylsilyl bromide was added. After 5 h the mixture was evaporated and purified with RPLC ($\text{CH}_3\text{CN}/\text{NH}_4\text{OAc}$ (0.1M), 30:70) to give the final compound after filtration, evaporation and freeze drying in 8 % yield.

15 $^1\text{H-NMR}$ (500 MHz, MeOD): δ 0.8-1.1 (m, 2H), 1.15-1.4 (m, 4H), 1.5-1.9 (m, 10H), 1.9-2.1 (m, 4H) 1.96 (s, acetate), 2.20 (m, 1H), 2.95 (m, 1H), 3.0-3.2 (m, 3H), 3.4-3.5 (m, 2H), 4.09 (m, 1H), 4.39 (bd, 1H), 4.59 (m, 1H).
20 $^{13}\text{C-NMR}$ (125 MHz, MeOD): guanidine: δ 158.6; carbonyl carbons: δ 174.2, 170.6

Example 52

25 $\text{EtO}(\text{HO})\text{P}(\text{O})-\text{CH}_2-(\text{R})\text{Cha-Pro-Nag} \times 2 \text{ HOAc}$

(i) $(\text{EtO})(\text{HO})\text{PO-CH}_2-(\text{R})\text{Cha-Pro-Nag(Z)}$.
30 ($\text{EtO})_2\text{PO-CH}_2-(\text{R})\text{Cha-Pro-Nag(Z)}$ (See Example 53), 50 mg (77 mmol) was dissolved in 2 ml of EtOH and 2 ml 2 M NaOH. The mixture was stirred over night and evaporated. The crude material was purified with RPLC ($\text{CH}_3\text{CN}/\text{NH}_4\text{OAc}$ (0.1M), 30:70) to give the title compound after filtration and evaporation
35 of the solvent.

(ii) $(\text{EtO})(\text{HO})\text{PO-CH}_2-(\text{R})\text{Cha-Pro-Nag} \times 2 \text{ HOAc}$

Prepared by using deprotection procedure (c) on the product (i) above.

5 $^1\text{H-NMR}$ (500 MHz, MeOD): δ 0.9-1.1 (m, 2H), 1.15-1.35 (m, 6H);
thereof 1.28 (t, 3H)), 1.35-1.5 (m, 2H), 1.5-1.6 (m, 1H),
1.65-1.8 (m, 6H), 1.9-2.1 (m, 3H) 1.95 (s, acetate), 2.19 (m,
1H), 2.8-3.0 (m, 2H), 3.1-3.25 (m, 2H), 3.27 (m, 1H), 3.36
(m, 1H), 3.48 (m, 1H), 3.9-4.05 (m, 4H), 4.36 (dd, 1H).

10 $^{13}\text{C-NMR}$ (125 MHz, MeOD): guanidine: δ 158.6; carbonyl
carbons: δ 175.0, 174.7

Example 53

15 $(\text{EtO})_2\text{P}(\text{O})-\text{CH}_2-(\text{R})\text{Cha-Pro-Nag} \times \text{HOAc}$

(i) $(\text{EtO})_2\text{PO}-\text{CH}_2-(\text{R})\text{Cha-Pro-Nag}(\text{Z})$.

20 H- (R) Cha-Pro-Nag (Z) (See Example 20), 0.2 g (0.40 mmol), was dissolved in 5 ml of THF and 0.11 g (0.80 mmol) of potassium carbonate and 0.12 g (0.40 mmol) diethyl triflylmethyl-phosphonate were added. The mixture was stirred at room temperature for 2 h. The reaction was worked up with water and extraction of the aqueous layer three times with EtOAc.
25 The combined organic layer was dried (Na_2SO_4) and evaporated to yield 0.14 g (53%) of the title compound.

(ii) $(\text{EtO})_2\text{PO}-\text{CH}_2-(\text{R})\text{Cha-Pro-Nag} \times \text{HOAc}$

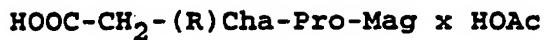
30 Prepared by using the deprotection procedure (c) on the product (i) above.

35 $^1\text{H-NMR}$ (500 MHz, MeOD): δ 0.85-1.05 (m, 2H), 1.15-1.3 (m,
5H), 1.34 (t, 6H), 1.5-1.85 (m, 8H), 1.9-2.05 (m, 3H) 1.91
(s, acetate), 2.10 (m, 1H), 2.22 (m, 1H), 2.90 (dd, 1H), 3.05
(dd, 1H), 3.1-3.3 (m, 3H), 3.42 (m, 1H), 3.53 (m, 1H), 3.71
(dd, 1H), 3.82 (m, 1H), 4.1-4.2 (m, 4H), 4.28 (dd, 1H).

^{13}C -NMR (125 MHz, MeOD), guanidine: δ 158.7; carbonyl carbons: δ 176.1, 175.1.

Example 54

5



(i) $\text{H-(R) Cha-Pro-NH-(CH}_2)_2\text{-NH(Z)}$

10 Prepared from Boc-(R)Cha-Pro-OSu and $\text{H}_2\text{N-(CH}_2)_2\text{-NH(Z)}$ in the same way as described for $\text{H-(R)Cha-Pro-Agm(Z)}$ in Example 3.

(ii) $\text{EtOOC-CH}_2\text{-(R)Cha-Pro-NH-(CH}_2)_2\text{-NH}_2 \times \text{HOAc}$

15 Alkylation as in Example 4 followed by deprotection procedure (a) gave the title compound.

(iii) $\text{HOOC-CH}_2\text{-(R)Cha-Pro-Mag} \times \text{HOAc}$

20 Guanidation of the amine above in the same way as described in Example 19 for Z-(R)Cha-Pro-Nag followed by deprotection procedure (e) gave the title compound after purification by RPLC ($\text{CH}_3\text{CN}/\text{NH}_4\text{OAc}$ (0.1M), 1/4) and freeze drying (H_2O).

25 $^1\text{H-NMR}$ (300 MHz, D_2O): δ 0.90-1.18 (m, 2H), 1.19-1.43 (m, 3H), 1.52 (m, 1H), 1.63-2.20 (m, 10H) 2.06 (s, acetate), 2.31-2.47 (m, 1H), 3.44 (m, 2H), 3.50 (m, 2H), 3.60-3.75 (m, 3H), 3.85 (m, 1H), 4.46-4.54 (m, 2H).

30 $^{13}\text{C-NMR}$ (75 MHz, D_2O): guanidine: δ 157.82; carbonyl carbons: δ 168.80, 171.41, 174.81.

Example 55

35 $\text{H-(R,S)Pro(3-Ph)-Pro-Agm} \times 2 \text{ TFA}$

Prepared from Boc-(R,S)Pro(3-Ph)-Pro-OSu (See Prep. of

starting materials) in the same way as described for H-(R)Cha-Pro-Agm(Z) in Example 3 followed by deprotection procedure (b).

5 $^1\text{H-NMR}$ (500 MHz, D_2O , mixture of two diastereomers with unknown relative stereochemistry): δ 1.0-1.8 (m, 7H), 2.0-2.5 (m, 3H), 2.8-4.3 (m, 10H), 4.56 (d, 1H, major), 4.90 (d, 1H, major), 7.2-7.5 (m, 5H).

10 $^{13}\text{C-NMR}$ (125.76 MHz, D_2O): guanidine: δ 157.36 (minor and major); carbonyl carbons: δ 174.1 (major), 174.0 (minor), 167.8 (major), 167.0 (minor).

Example 56

15 $\text{H-(R,S)Pro(3-(trans)Ch)-Pro-Agm} \times 2 \text{ TFA}$

Prepared from Boc-(R,S)Pro(3-(trans)Ch)-Pro-OSu (See Prep. of starting materials) in the same way as described for
20 H-(R)Cha-Pro-Agm(Z) in Example 3 followed by deprotection procedure (b).

1 $^1\text{H-NMR}$ (500 MHz, D_2O , mixture of two diastereomers, ratio 1.8/1): δ 0.95-1.32 (m 5H), 1.35-1.46 (m, 1H), 1.50-1.92 (m, 10H), 1.93-2.15 (m, 4H), 2.23-2.43 (m, 2H), 3.15-3.30 (m, 4H), 3.35-3.50 (m, 2H), 3.57-3.68 (m, 1H), 3.74-3.82 (m, 1H), 4.34-4.41 (m, 1H), 4.51 (d, 1H, minor), 4.48 (d, 1H, major).

1 $^{13}\text{C-NMR}$ (125.76 MHz, D_2O): guanidine: δ 157.36 (minor and major), carbonyl carbons: δ 174.34 (major), 174.07 (minor), 168.94 (minor and major).

Example 57

35 $\text{HOOC-CH}_2\text{-(R,S)Pro(3-(trans)Ph)-Pro-Agm} \times 2 \text{ TFA}$

(i) $\text{H-(R,S)Pro(3-(trans)Ph)-Pro-Agm(Z)}$

Prepared from Boc-(R,S)Pro(3-(trans)Ph)-Pro-OSu (See Prep. of starting materials) in the same way as described for H-(R)Cha-Pro-Agm(Z) in Example 3.

5 (ii) HOOC-CH₂-(R,S)Pro(3-(trans)Ph)-Pro-Agm x 2 TFA

Alkylation as in Example 4 using Br-CH₂COOBn followed by deprotection procedure (b) gave the title compound as a mixture of two diastereomers.

10

¹H-NMR (500 MHz, MeOD, mixture of two diastereomers, ratio ca: 1.1/1): δ 1.40-1.80 (m, 6H), 1.85-2.05 (m, 1H), 2.10-2.30 (m, 1H), 2.50-2.65 (m, 2H), 3.10-3.40 (m, 6H), 3.50-3.70 (m, 2H), 3.9-4.40 (m, 4H), 4.63 (d, 1H, major), 4.67 (d, 1H, minor), 7.30-7.60 (m, 5H).

15

¹³C-NMR (125.76 MHz, D₂O): guanidine: δ 157.52 (both isomers); carbonyl carbons: δ 173.87, 173.73, 169.12, 168.94, 167.21, 167.00.

20

Example 58

HOOC-CH₂-(R,S)Pro(3-(trans)Ph)-Pro-Nag x 2 TFA

25 (i) H-(R,S)Pro(3-(trans)Ph)-Pro-Nag(Z)

Prepared from Boc-(R,S)Pro(3-(trans)Ph)-Pro-OSu (See Prep. of starting materials) and Boc-Nag(Z) in the same way as described for H-(R)Cha-Pro-Agm(Z) in Example 3.

30

(ii) HOOC-CH₂-(R,S)Pro(3-(trans)Ph)-Pro-Nag x 2 TFA

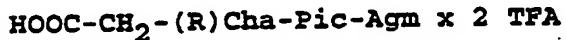
Alkylation as in Example 4 using Br-CH₂COOBn followed by deprotection procedure (b) gave the title compound as a mixture of two diastereomers.

¹H-NMR (500 MHz, MeOD, mixture of two diastereomers, ratio

ca: 1.5/1): δ 1.40-1.85 (m, 4H), 1.90-2.00 (m, 1H), 2.10-2.31
(m, 1H), 2.45-2.70 (m, 2H), 3.08-3.46 (m, 6H), 3.57-3.70 (m,
2H), 3.90-4.0 (m, 1H), 4.32-4.40 (m, 1H), 4.04 and 4.29
(AB-quartet, 2H, major), 4.16 and 4.37 (AB-quartet, 2H,
minor), 4.60 (d, 1H, major), 4.64 (d, 1H, minor), 7.3-7.5 (m,
5H).

^{13}C -NMR (125.76 MHz, D_2O): guanidine: δ 157.48 (both
isomers); carbonyl carbons: δ 173.90, 173.71, 169.01, 168.34,
10 167.07 (both isomers).

Example 59



15

(i) $\text{H-(R)Cha-Pic-Agm(Z)}$

Prepared from Boc-(R)Cha-Pic-OSu (See Prep. of starting
materials) in the same way as described for

20 $\text{H-(R)Cha-Pro-Agm(Z)}$ in Example 3.

(ii) $\text{HOOC-CH}_2\text{-(R)Cha-Pic-Agm} \times 2 \text{ TFA}$

Alkylation as in Example 4 using $\text{Br-CH}_2\text{COOBn}$ followed by
25 deprotection procedure (a) gave the title compound.

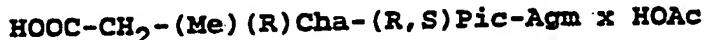
$^1\text{H-NMR}$ (300 MHz, MeOD): δ 1.02 (m, 2H), 1.13-2.00 (m, 20H),
2.24 (bd, 1H), 3.12-3.45 (m, 5H), 3.71 (bd, 1H), 3.87 (s,
2H), 4.65 (bt, 1H), 5.06 (m, 1H).

30

$^{13}\text{C-NMR}$ (75 MHz, D_2O): guanidine: δ 157.47; carbonyl carbons:
 δ 169.42, 170.03, 172.71.

Example 60

35



(i) Me-(R)Cha-(R,S)Pic-Agm(Z)

Prepared from Boc-(Me)(R)Cha-Pic-OSu in the same way as described for H-(R)Cha-Pro-Agm(Z) in Example 3.

5

(ii) HOOC-CH₂-(Me)(R)Cha-(R,S)Pic-Agm x HOAc

Alkylation as in Example 4 using Br-CH₂COOBn followed by deprotection procedure (b) gave the title compound.

10

Comment: An epimerization of Pic occurred somewhere during the synthesis.

The ¹H-NMR spectrum is complex consisting of two diastereomers ca: 1:1 ratio and rotamers thereof.

¹H-NMR (500 MHz, MeOD): δ 0.75-2.15 (several m, 20H) 1.95 (bs, acetate), 2.2-2.7 (6H, two distinct sets of signals are observed in the ratio of ca: 1:1; thereof 2.35 and 2.55 (s, 20 3H)), 3.0-3.5 (m, 6H), 3.9-4.17 (m, 2H; thereof 4.14 (dd)), 4.4-4.5 (m, 1H), 4.97-5.15 (two bdd, 1H).

¹³C-NMR (75MHz, D₂O): guanidine: δ 157.50; carbonyl carbons: δ 169.65, 170.01, 170.54, 172.67, 172.89.

25

Example 61**HOOC-(R,S)CH(Me)-(R)Cha-Pic-Agm x TFA**

30 Alkylation as in Example 4 using H-(R)Cha-Pic-Agm(Z) (See Example 59), and Br-CH(Me)COOBn followed by deprotection procedure (a) gave the title compound as a mixture of two diastereomers.

35 Example 62**HOOC-(RorS)CH(Me)-(R)Cha-Pic-Agm/a x 2 TFA**

Obtained by separating the diastereomers formed in Example 61 using RPLC ($\text{CH}_3\text{CN}/\text{NH}_4\text{OAc}$ (0.1M), 1/3) followed by evaporation of the solvent and freeze-drying from $\text{H}_2\text{O}/\text{TFA}$. This diastereomer came out first of the two from the column.

5

$^1\text{H-NMR}$ (300 MHz, D_2O , 2 rotamers ca: 5:1 ratio): δ 0.70 (m, minor rotamer), 0.75-1.0 (m, 2H), 1.0-1.28 (m, 3H), 1.28-1.83 (m, 20H; thereof 1.57 (d, 3H)), 2.14 (bd, 1H), 2.92 (t, minor rotamer), 3.03-3.32 (m, 5H), 3.59 (bd, 1H), 3.85 (q, minor rotamer), 3.98 (q, 1H), 4.30-4.50 (m, minor rotamer), 4.54 (m, 1H), 4.95 (s, 1H).

10

$^{13}\text{C-NMR}$ (75 MHz, D_2O): guanidine: δ 157.39; carbonyl carbons: δ 172.26 (2 carbons), 169.92.

15

Example 63

HOOC-(RorS)CH(Me)-(R)Cha-Pic-Agm/b x 2 TFA

20 The title compound was obtained by using the same procedure as described in Example 62 on the compound formed in Example 61. This diastereomer came out after the first one from the column.

25 $^1\text{H-NMR}$ (500 MHz, D_2O , 2 rotamers ca: 5:1 ratio): δ 0.72 (m, minor rotamer), 0.82 (m, minor rotamer), 0.97 (m, 2H), 1.0-1.23 (m, 3H), 1.23-1.40 (m, 2H), 1.40-1.83 (m, 18H; thereof 1.63 (d, 3H)), 2.11 (d, 1H), 2.17 (d, minor rotamer), 2.92 (t, minor rotamer), 3.05-3.25 (m, 4H), 3.29 (t, 1H), 3.74 (d, 1H), 4.02 (q, 1H), 4.34 (d, minor rotamer), 4.41 (dd, minor rotamer), 4.52 (t, 1H), 4.95 (s, 1H).

30

$^{13}\text{C-NMR}$ (125 MHz, D_2O): guanidine: δ 154.68; carbonyl carbons: δ 169.31, 169.60, 167.36.

35

Example 64

HOOC-CH₂-CH₂-(R)Cha-Pic-Agm x 2 TFA

Prepared from H-(R)Cha-Pic-Agm(Z) (See Example 59) in the same way as described for HOOC-CH₂-CH₂-(R)Cha-Pro-Agm in

5 Example 15 using 1.2 eq. of benzylacrylate instead of 1.1 eq.

15 ¹H-NMR (500 MHz, D₂O, 2 rotamers ca: 4:1 ratio): δ 0.70-0.90 (m, minor rotamer), 0.90-1.0 (m, 2H), 1.05-1.25 (m, 3H), 1.30-1.45 (m, 2H), 1.45-1.85 (m, 15H), 2.1 (bd, 1H), 2.2 (bd, 10 minor rotamer), 2.75 (t, 2H), 2.95 (t, minor rotamer), 3.1-3.4 (m, 7H), 3.75 (bd, 1H), 4.55 (t, 1H), 4.95 (m, 1H).

13C-NMR (75 MHz, D₂O): guanidine: δ 157.48; carbonyl carbons: δ 170.10, 172.58, 174.75.

15

Example 65**H-(R)Cha-Pic-Nag x 2 TFA**

20 (i) Boc-(R)Cha-Pic-Nag(Z)

(ia) Prepared by starting from Boc-(R)Cha-Pic-OSu by using the same procedure as described for Boc-(R)Cha-Pro-Agm(Z) in Example 3.

25

(ib) Prepared by starting from Boc-(R)Cha-Pic-OH

Diphenylphosphoryl azide (0.432 ml, 2 mmol) was added to a stirred solution of Boc-(R)Cha-Pic-OH (765 mg, 2 mmol) in 5 30 ml DMF at -10 °C. After 10 minutes H-Nag(Z) x 2 HCl (600 mg, 2.1 mmol, see Preparation of Starting Materials) in 5 ml DMF and triethylamine (615 mg, 4.4 mmol) was added. The reaction mixture was kept in an ice bath for 3 h and then at room temperature for 12 h after which it was poured out in water. 35 Extraction of the water phase with EtOAc followed by drying (MgSO₄) of the organic phase and evaporation of the solvent in vacuo gave 1.18 g (96 %) of the product as a mixture of

diastereomers (Epimers in Pic) in a ratio of 97:3 (RS/RR).

(ic) Starting from Boc-(R)Cha-Pic-OH

5 EDC hydrochloride (4.2 g, 21.9 mmol) was added at -15° C to a stirred solution of Boc-(R)Cha-Pic-OH (8 g, 20.9 mmol), DMAP (10.6 g, 88 mmol) and H-Nag-(Z) x 2 HCl (6.3 g, 19.5 mmol, see Preparation of Starting Materials) in acetonitrile. The reaction mixture was allowed to warm up to +15° C during 16
10 h. The solvent was removed in vacuo and the residue was dissolved in ethyl acetate. Washing with water, 0.3 M KHSO₄, 0.3 M NaHCO₃, water and brine followed by drying (Na₂SO₄) and evaporation of the solvent gave 11.9 g (92.5%) of the product as a mixture of diastereomers (Epimers in Pic) in a ratio of
15 98/2 (RS/RR).

¹H-NMR (500 MHz, CDCl₃): δ 0.85-2.0 (m, 29H; thereof 1.40 (bs, 9H)), 2.46 (bd, 1H), 3.1-3.4 (m, 5H), 3.92 (bd, 1H), 4.53 (bq, 1H), 5.10 (s, 2H), 5.22 (bs, 1H), 5.29 (bd, 1H), 6.7-7.2
20 (b, 3H), 7.25-7.45 (m, 5H).

¹³C-NMR (125 MHz, CDCl₃): guanidine δ 156.9; carbonyl carbons: δ 173.6, 170.3, 163.7, 161.7.

25 (ii) H-(R)Cha-Pic-Nag(Z)

Prepared in the same way as described for H-(R)Cha-Pro-Agm(Z) in Example 3, starting from Boc-(R)Cha-Pic-Nag(Z).

30 ¹H-NMR (500 MHz, CDCl₃): δ 0.8-2.0 (m, 22H), 2.24 (bd, 1H), 3.1-3.4 (m, 5H), 3.72 (bd, 1H), 3.84 (bq, 1H), 5.05 (bd, 1H), 5.08 (s, 2H), 7.3-7.5 (m, 5H).

(iii) H-(R)Cha-Pic-Nag x 2 TFA

35

Prepared by using the deprotection procedure (a) on the product (ii) above.

¹H-NMR (500 MHz, MeOD): δ 0.9-1.1 (m, 2H), 1.2-2.0 (m, 18H), 2.32 (bd, 1H), 3.20 (t, 2H), 3.30 (t, 2H), 3.36 (m, 1H), 3.63 (bd, 1H), 4.49 (dd, 1H), 5.05 (bd, 1H).

5 ¹³C-NMR (125 MHz, MeOD): guanidine: δ 158.7; carbonyl carbons: δ 172.7, 171.4

Example 66

10 Me-(R)Cha-(R,S)Pic-Nag x 2 TFA

(i) Me-(R)Cha-(R,S)Pic-Nag(Z)

Prepared in the same way as described for H-(R)Cha-Pro-Agm(Z)
15 in Example 3 starting from Boc-(Me)(R)Cha-Pic-OSu and Boc-
Nag(Z). An epimerization of Pic occurred during the synthesis
and the product was obtained as mixture of two diastereomers.

(ii) Me-(R)Cha-(R,S)Pic-Nag x 2 TFA

20

Prepared by using deprotection procedure (b).

The ¹H-NMR spectrum is complex consisting of two diastereomers ca: 4:1 ratio and rotamers thereof.

25

¹H-NMR (500 MHz, MeOD): δ 0.8-1.08 (m, 2H), 1.15-2.4 (several m, 19H), 2.6-2.75 and 2.9-2.95 (several s, 3H) 3.1-3.6 (several m, 5H), 3.75-4.1 (several m, 1H) 4.4-4.7 (several m, 1H), 5.05-5.15 (two dd, 1H).

30

¹³C-NMR (125 MHz, D₂O): guanidine: δ 154.84; carbonyl carbons: δ 167.60 and 169.99.

Example 67

35

HOOC-CH₂-(R)Cha-Pic-Nag

(i) $\text{BnOOC-CH}_2\text{-(R)Cha-Pic-Nag(Z)}$

Alkylation as in Example 4 using H-(R)Cha-Pic-Nag(Z) (See Example 65) and Br- CH_2COOBn gave the title compound.

5

$^1\text{H-NMR}$ (500 MHz, CDCl_3): δ 0.8-1.0 (m, 2H), 1.1-1.7 (m, 19H), 1.79 (bd, 1H), 2.3-2.5 (m, 2H; thereof 2.38 (bd, 1H)), 3.00 (bt, 1H), 3.1-3.4 (m, 5H; thereof 3.38 (d, 1H)) 3.58 (d, 1H), 3.6-3.7 (m, 2H), 5.06 (dd, 2H), 5.07 (s, 2H), 5.16 (bs, 1H), 10 6.7-7.1 (b, 1H), 7.15 (bs, 1H), 7.2-7.4 (m, 10H).

$^{13}\text{C-NMR}$ (125 MHz, CDCl_3) guanidine and carbonyl carbons: δ 176.0, 173.6, 170.8, 163.8, 161.7.

15 (iia) $\text{HOOC-CH}_2\text{-(R)Cha-Pic-Nag} \times 2 \text{ HCl}$

Deprotection procedure (a) followed by purification with RP LC using $\text{CH}_3\text{CN}/0.1 \text{ M NH}_4\text{OAc}$, 1/3 as eluent, evaporation at 40-50° C and freeze drying gave the title compound as the acetate. Treatment with a 20-fold excess of hydrochloric acid, evaporation and renewed freeze drying gave the bis-hydrochloride of the desired compound.

25 $^1\text{H-NMR}$ (500MHz, D_2O , mixture of two rotamers) : δ 0.7-2.0 (m, 20H), 2.17 (bd, 1H), 2.95 (t, minor rotamer), 3.17 (t, 2H), 3.25-3.35 (m, 3H), 3.72 (bd, 1H), 3.86 (dd, minor rotamer), 3.90 (s, 2H), 4.72 (t, 1H), 4.99 (bs, 1H).

30 $^{13}\text{C-NMR}$ (75 MHz, D_2O); guanidine δ 157.4; carbonyl carbons δ 169.9, 170.2, 173.0.

(iib) $\text{HOOC-CH}_2\text{-(R)Cha-Pic-Nag} \times 2 \text{ HBr}$

35 $\text{BnOOC-CH}_2\text{-(R)Cha-Pic-Nag(Z)}$ was dissolved in $^i\text{Pr-OH/H}_2\text{O}$ (95/5) and hydrogenated over 5% Pd/C at atmospheric pressure in the presence of HBr (2.2 eq.). The catalyst was filtered off and the solvent evaporated to give a yellow oil

(Alternatively, the acid can be added after hydrogenation and filtration). Crystallisation from i Pr-OH (or EtOH)/EtOAc (1/1) gave the title compound as a white crystalline powder.

5 $^1\text{H-NMR}$ (500 MHz, D_2O , mixture of two rotamers): δ 1.15-2.0 (m, 20H), 2.30 (bd, 1H), 3.30 (m, 2H), 3.40-3.50 (m, 3H), 3.85-3.90 (m, 1H), 3.95 (apparent s, 2H), 4.75-4.85 (m, 1H, partially hidden by the H-O-D line), 5.10 (bs, 1H).

10 $^{13}\text{C-NMR}$ (125 MHz, D_2O): guanidine: δ 157.6; carbonyl carbons: δ 169.7, 170.2, 173.0.

Example 68

15 **MeOOC-CH₂-(R)Cha-Pic-Nag x 2 TFA**

The methyl ester MeOOC-CH₂-(R)Cha-Pic-Nag(Z) was obtained by trans esterification of i PrOOC-CH₂-(R)Cha-Pic-Nag(Z) (See Example 69) on the column during flash chromatography when 20 $\text{CH}_2\text{Cl}_2/\text{MeOH}$ was used as eluent. The title compound was obtained by the deprotection procedure (a).

25 $^1\text{H-NMR}$ (500 MHz, MeOD): δ 0.95-1.15 (m, 2H), 1.2-1.6 (m, 6H), 1.65-2.0 (m, 13H), 2.25 (bd, 1H), 3.21 (t, 2H), 3.30 (t, 2H), 3.37 (m, 1H), 3.71 (m, 1H), 3.83 (s, 3H), 3.97 (dd, 2H), 4.67 (bt, 1H), 5.05 (bs, 1H).

30 $^{13}\text{C-NMR}$ (125 MHz, MeOD), guanidine: δ 158.0; carbonyl carbons: δ 173.0, 171.1, 168.3.

Example 69

i PrOOC-CH₂-(R)Cha-Pic-Nag x 2 TFA

35 Alkylation as described in Example 4 using H-(R)Cha-Pic-Nag(Z) (See Example 65) and Br-CH₂-COO i Pr followed by deprotection procedure (a) gave the title

compound.

5 $^1\text{H-NMR}$ (500 MHz, MeOD): δ 0.95-1.1 (m, 2H), 1.15-1.6 (m, 12H; thereof 1.25 (d, 3H), 1.28 (d, 3H)), 1.65-1.95 (m, 12H), 2.28 (bd, 1H), 3.21 (t, 2H), 3.30 (t, 2H), 3.36 (m, 1H), 3.93 (dd, 2H), 4.67 (t, 1H), 5.04 (bs, 1H), 5.11 (pentet, 1H).

10 $^{13}\text{C-NMR}$ (125 MHz, MeOD), guanidine: δ 157.9; carbonyl carbons: δ 173.1, 171.0, 168.3.

10

Example 70



15 Alkylation as described in Example 4 using Me-(R)Cha-(R,S)Pic-Nag(Z) (See Example 66) and Br-CH₂-CCOBn followed by deprotection procedure (b) gave HOOC-CH₂-(Me)(R)Cha-(R,S)Pic-Nag. The two diastereomers were separated by RPLC (CH₃CN/NH₄OAc, 1:3) followed by 20 freeze-drying from H₂O/TFA. This diastereomer came out last of the two from the column.

25 $^1\text{H-NMR}$ (500 MHz, MeOD): δ 0.9-1.1 (m, 2H), 1.15-1.35 (m, 4H), 1.4-1.55 (m, 2H), 1.6-1.85 (m, 12H), 2.3 (m, 1H), 2.85 (s, 3H), 3.15-3.45 (m, 5H), 3.65 (bs, 2H), 4.0 (m, 1H), 4.65 (m, 1H), 5.08 (dd, 1H).

30 $^{13}\text{C-NMR}$ (75 MHz, D₂O): guanidine: δ 157.65; carbonyl carbons: δ 169.86 and 172.48.

30

Example 71



35 Alkylation as described in Example 4 using H-(R)Cha-Pic-Nag(Z) (See Example 65) and Br-CH(Me)-COOBn followed by deprotection procedure (a) gave the title compound as a

mixture of four diastereomers.

Example 72

5 HOOC-(RorS)CH(Me)-(R)Cha-(RorS)Pic-Nag/c x 2 TFA

Obtained by separating the diastereomers formed in Example 71 using RPLC (CH₃CN/NH₄OAc (0.1M), 1/4) followed by evaporation and freeze-drying from H₂O/TFA. This diastereomer came out as
10 the third one of the four from the column.

15 ¹H-NMR (300 MHz, D₂O, 2 rotamers ca: 5:1 ratio): δ 0.88 (m, minor rotamer), 0.98-1.63 (m, 7H), 1.63-2.02 (m, 16H; thereof 1.68 (d, 3H), 2.28 (m, 1H), 3.10 (t, minor rotamer), 3.25-3.50 (m, 5H; thereof 3.33 (t, 2H) and 3.43 (t, 2H)), 3.82 (bd, 1H), 4.02 (q, 1H), 4.55 (d, minor rotamer), 4.65 (d, minor rotamer), 4.72 (m, 1H), 5.10 (m, 1H).

Example 73

20

HOOC-(RorS)CH(Me)-(R)Cha-(RorS)Pic-Nag/d x 2 TFA

Obtained by separating the diastereomers formed in Example 71 using RPLC (CH₃CN/NH₄OAc (0.1 M), 1:4) followed by
25 evaporation and freeze-drying from H₂O/TFA. This diastereomer came out last of the four diastereomers from the column.

30 ¹H-NMR (500 MHz, D₂O, 2 rotamers ca: 5:1 ratio): δ 0.80 (m, minor rotamer), 0.90 (m, minor rotamer), 1.03 (m, 2H), 1.10-1.33 (m, 3H), 1.42 (m, 2H), 1.51-1.92 (m, 16H; thereof 1.57 (d, 3H)), 2.18 (d, 1H), 2.24 (d, minor rotamer), 2.98 (t, minor rotamer), 3.21 (t, 2H), 3.28-3.40 (m, 3H; thereof 3.44 (t, 2H)), 3.82 (d, 1H), 4.02 (q, 1H), 4.42 (d, minor rotamer), 4.50 (t, minor rotamer), 4.62 (t, 1H), 4.67 (s, minor rotamer), 5.03 (s, 1H).

Example 74

HOOC-CH₂-CH₂-(R)Cha-Pic-Nag x 2 TFA

Prepared from H-(R)Cha-Pic-Nag(Z) (See Example 65) in the same way as described for HOOC-CH₂-CH₂-(R)Cha-Pro-Agm in
5 Example 15 using 1.2 eq. of benzylacrylate instead of 1.1 eq.

1H-NMR (500 MHz, D₂O, 2 rotamers ca: 4:1 ratio): δ 0.7-0.9 (m, minor rotamer), 0.9-1.0 (m, 2H), 1.05-1.3 (m, 3H), 1.3-1.45 (m, 2H), 1.5-1.8 (m, 13H), 2.10 (d, 1H), 2.20 (d, 10 minor rotamer), 2.75 (t, 2H), 2.95 (t, minor rotamer), 3.15 (t, 2H), 3.2-3.35 (m, 5H), 3.75 (d, 1H), 4.55 (t, 1H), 4.95 (m, 1H).

13C-NMR (75 MHz, D₂O): guanidine: δ 157.57; carbonyl carbons:
15 δ 170.16, 172.82, 174.75.

Example 75**HOOC-CH₂-(R)Cha-(R,S)Mor-Agm x 2 TFA**

20 (i) H-(R)Cha-Mor-Agm(Z)

Prepared from Boc-(R)Cha-Mor-OSu (See Prep. of starting materials) in the same way as described for
25 H-(R)Cha-Pro-Agm(Z) in Example 3.

(ii) HOOC-CH₂-(R)Cha-(R,S)Mor-Agm x 2 TFA

Alkylation as in Example 4 using Br-CH₂COOBn followed by
30 deprotection procedure (b) gave the title compound. An epimerization of Mor had occurred somewhere during the synthesis and a mixture of about 9:1 of two diastereomers was observed in the final product.

35 1H-NMR (300 MHz, MeOD): δ 0.92-1.95 (m, 17 H), 3.12-3.39 (m, 4H), 3.44-4.05 (m, 7H), 4.37 (d, 1H), 4.63 (m, 1H), 4.79 (bd, 1H).

^{13}C -NMR (75.47 MHz, MeOD): guanidine: δ 158.63; carbonyl carbons: δ 170.87, 170.82, 169.08 others: δ 69.06, 67.01 ($\underline{\text{C}}\text{-O-}\underline{\text{C}}$).

5 Example 76



(i) H-(R)Cha-Mor-Nag(Z)

10

Prepared from Boc-(R)Cha-Mor-OSu (See Prep. of starting materials) and Boc-Nag(Z) in the same way as described for H-(R)Cha-Pro-Agm(Z) in Example 3.

15 (ii) $\text{HOOC-CH}_2\text{-(R)Cha-(RorS)Mor-Nag} \times 2 \text{ TFA}$

Alkylation as described in Example 4 using $\text{Br-CH}_2\text{COOBn}$ followed by deprotection procedure (b) gave the title compound.

20

$^1\text{H-NMR}$ (300 MHz, MeOD): δ 0.92-1.13 (m, 2H), 1.15-1.42 (m, 3H), 1.50 (br.s, 1H), 1.62-1.95 (m, 9H), 3.14-3.40 (m, 4H), 3.46-4.13 (m, 7H), 4.41 (d, 1H), 4.63 (m, 1H), 4.80 (br.d, 1H).

25

$^{13}\text{C-NMR}$ (75.47 MHz, MeOD): guanidine: δ 158.68; carbonyl carbons: δ 171.19, 170.90, 169.46. others: δ 68.81, 67.00 ($\underline{\text{C}}\text{-O-}\underline{\text{C}}$).

30 Example 77



(i) Boc-(R)Cha-Aze-Nag(Z)

35

Prepared from Boc-(R)Cha-Aze-OH in the same way as described for Boc-(R)Cha-Pic-Nag(Z) according to Example 65 (ic).

(ii) H-(R)Cha-Aze-Nag(Z)

Prepared in the same way as described for H-(R)Cha-Pro-Agm(Z).
(See Example 3).

5

(iii) H-(R)Cha-Aze-Nag x 2 HOAc

Prepared by using the deprotection procedure (a) on the product (ii) above.

10

¹H-NMR (300 MHz, D₂O): δ 0.85-1.10 (m, 2H), 1.10-2.04 (m, 13H) 1.95 (s, acetate), 2.20-2.37 (m, 1H), 2.60-2.82 (m, 1H), 3.15-3.40 (m, 4H), 3.96-4.15 (m, 2H), 4.18-4.30 (m, 1H), 4.30-4.42 (m, 1H), signals of a minor rotamer appears at: δ 15 0.70, 3.90 and 5.10.

¹³C-NMR (75 MHz, D₂O): guanidine: δ 157.39 and carbonyl carbons: δ 170.22 and 172.38.

20 Example 78HOOC-CH₂- (R)Cha-Aze-Nag x HOAc(i) BnOOC-CH₂- (R)Cha-Aze-Nag(Z)

25

Prepared from H-(R)Cha-Aze-Nag(Z) (See Example 77) according to the procedure described in Example 4.

30 (ii) HOOC-CH₂- (R)Cha-Aze-Nag x HOAc

Prepared by using the deprotection (a) on the product (i) above.

35

¹H-NMR (500 MHz, MeOD): δ 0.90-1.10 (m, 2H), 1.15-2.00 (m, 13H) 1.95 (s, acetate), 2.20-2.30 (m, 1H), 2.58-2.70 (m, 1H), 3.17-3.30 (m, 4H), 3.35-3.50 (m, 2H), 3.55-3.68 (m, 1H),

4.10-4.20 (m, 1H), 4.30-4.38 (m, 1H), 4.65-4.77 (m, 1H), signals of minor rotamer appears at: δ 3.75, 3.98, 4.03 and 5.08.

5 ^{13}C -NMR (75 MHz, D_2O): guanidine: δ 157.40 and carbonyl carbons: δ 169.16, 171.92 and 172.13.

Example 79

10 H-(R)Cha-Pro(5-(S)Me)-Nag x 2 HCl

(i) Boc-(R)Cha-Pro(5-(S)Me)-Nag(Z)

The same procedure as described for the coupling between Boc-(R)Cha-OH and H-Pic-OEt x HCl (See Preparation of Starting Materials) was used to accomplish the coupling between Boc-(R)Cha-Pro(5-(S)Me)-OH and H-Nag(Z) x 2 HCl.

(ii) H-(R)Cha-Pro(5-(S)Me)-Nag(Z)

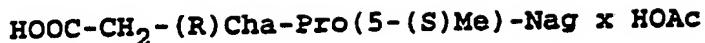
20 The same procedure as described for the synthesis of H-(R)-Cgl-Pic-Nag(Z) (See Example 84 (ii) was used.

(iii) H-(R)Cha-Pro(5-(S)Me)-Nag x 2 HCl

25 Prepared by using the deprotection procedure (d) on the product (ii) above.

30 ^1H -NMR (300 MHz, D_2O): δ 1.0-2.3 (m, 21H); thereof 1.47 (d, 3H), 2.4-2.55 (m, 1H), 3.3-3.6 (m, 4H), 4.30 (bt, 1H), 4.38 (dd, 1H), 4.47 (bt, 1H).

35 ^{13}C -NMR (75 MHz, D_2O): guanidine: δ 157.6 carbonyl carbons: δ 174.6, 169.6.

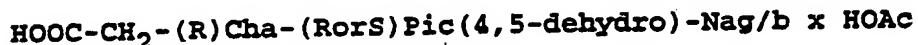
Example 80

5 Alkylation as in Example 4 using H-(R)Cha-Pro(5-(S)Me)-Nag(Z)
(See Example 79) and Br-CH₂-COOBn followed by deprotection
procedure (a) gave the title compound.

10 ¹H-NMR (300 MHz, D₂O): δ 0.9-1.9 (m, 19H); thereof 1.34 (bd,
3H), 1.93 (s, acetate), 2.0-2.2 (m, 3H), 2.34 (m, 1H), 3.1-
3.5 (m, 7H), 3.97 (m, 1H), 4.20 (m, 1H), 4.31 (bt, 1H).

15 ¹³C-NMR (75 MHz, D₂O): guanidine: δ 157.4.

15 Example 81



20 (i) Boc-(R)Cha-(R,S)Pic(4,5-dehydro)-Nag(Z)
Prepared from Boc-(R)Cha-(R,S)Pic(4,5-dehydro)-OH in the same
way as described for Boc-(R)Cha-Pic-Nag(Z) (See Example 65
(ic)).

25 (ii) H-(R)Cha-(R,S)Pic(4,5-dehydro)-Nag(Z)

Prepared in the same way as described for H-(R)Cha-Pro-Agm(Z)
(See Example 3).

30 (iii) BnOOC-CH₂-(R)Cha-(R,S)Pic(4,5-dehydro)-Nag(Z)

Prepared from H-(R)Cha-(R,S)Pic(4,5-dehydro)-Nag(Z) according
to the procedure described in Example 4.

35 (iv) HOOC-CH₂-(R)Cha-(R,S)Pic(4,5-dehydro)-Nag/b x HOAc

A mixture of 356 mg (0.539 mmol) of BnOOC-CH₂-(R)Cha-(R,S)

Pic(4,5-dehydro)-Nag(Z), 10.8 mL trifluoroacetic acid and 3.4 mL tioanisole was stirred at room temperature for 3.5 h. Water was added and the mixture was washed twice with CH_2Cl_2 . Evaporation of the solvent gave HOOC- CH_2 -(R)Cha-(R,S)Pic(4,5-dehydro)-Nag. The title compound was obtained by separating the diastereomers by RPLC ($\text{CH}_3\text{CN}/\text{NH}_4\text{OAc}$ (0.1 M), 3/7) and freeze drying (H_2O) after evaporation of the solvent. The diastereomer came out last of the two from the column.

$^1\text{H-NMR}$ (300 MHz, D_2O) δ 0.85-1.95 (m, 15H), 2.50-2.80 (m, 2H), 3.25 (t, 2H), 3.35 (t, 2H), 3.55 (bs, 2H), 3.85-4.6 (m, 3H), 4.92 (minor rotamer), 5.30 (d, 1H), 5.85-6.1 (m, 2H), $^{13}\text{C-NMR}$ (75 MHz, D_2O): guanidine: δ 157.59; carbonyl carbons: δ 171.46, 172.58, 173.03.

Example 82

HOOC-CH_2 -(R)Cha-Pic(4-(S)Me)-Nag x 2 HCl

(i) Boc-(R)Cha-Pic(4-(S)Me)-Nag(Z)

Prepared from Boc-(R)Cha-Pic(4-(S)Me)-OH in the same way as described for Boc-(R)Cha-Pic-Nag(Z) according to method (ic) in Example 65.

(ii) H-(R)Cha-Pic(4-(S)Me)-Nag(Z)

Prepared in the same way as described for H-(R)Cha-Pro-Agm(Z) (See Example 3).

(iii) BnOOC- CH_2 -(R)Cha-Pic(4-(S)Me)-Nag(Z)

Prepared from H-(R)Cha-Pic(4-(S)Me)-Nag(Z) according to the procedure described in Example 4.

(iv) HOOC-CH₂-(R)Cha-Pic(4-(S)Me)-Nag x 2 HCl

Prepared by using the deprotection procedure (d) on the product (iii) above.

5

¹H-NMR (500 MHz, D₂O): δ 0.95-2.05 (m, 22H; thereof 1.05 (d, 3H)), 2.30-2.38 (bd, 1H), 3.28-3.36 (m, 2H) 3.36-3.50 (m, 3H), 3.85-3.95 (m, 1H), 3.98 (s, 2H), 4.70-4.90 (m, 1H; partly hidden behind the HOD signal), 5.22-5.27 (d, 1H),

10 signal of a minor roatmer appears at δ 0.93, 3.13 and 4.57.

¹³C-NMR (125 MHz, D₂O): guanidine: δ 157.58; carbonyl carbons: δ 170.12, 170.32 and 172.82.

15 Example 83

HOOC-CH₂-(R)Cha-(R)Pic(4-(R)Me)-Nag x 2 HCl

(i) Boc-(R)Cha-(R)Pic(4-(R)Me)-Nag(Z)

20

Prepared from Boc-(R)Cha-(R)Pic(4-(R)Me)-OSu and Boc-Nag(Z) in the same way as described for Boc-(R)Cha-Pro-Agm(Z) (See Example 3).

25 (ii) H-(R)Cha-(R)Pic(4-(R)Me)-Nag(Z)

Prepared in the same way as described for H-(R)Cha-Pro-Agm(Z) (See Example 3).

30 (iii) BnOOC-CH₂-(R)Cha-(R)Pic(4-(R)Me)-Nag(Z)

Prepared from H-(R)Cha-(R)Pic(4-(R)Me)-Nag(Z) according to the procedure described in Example 4.

35 (iv) HOOC-CH₂-(R)Cha-(R)Pic(4-(R)Me)-Nag x 2 HCl

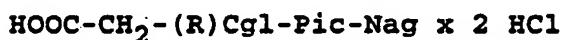
Prepared by using the deprotection procedure (d) on the

product (iii) above.

5 $^1\text{H-NMR}$ (500 MHz, D_2O): δ 1.00-2.05 (m, 22H), 2.18-2.26 (bd, 1H), 3.28-3.36 (m, 2H), 3.36-3.55 (m, 3H), 3.85-4.05 (m, 3H), 4.70-4.90 (m, 1H; partly hidden behind the HOD signal), 5.25-5.30 (d, 1H), signals of minor rotamer apppears at: δ 2.40, 2.90, 4.10, 4.42, 4.55 and 5.23.

10 $^{13}\text{C-NMR}$ (125 MHz, D_2O): guanidine: δ 157.56: carbonyl carbons: δ 169.69, 169.84 and 173.20.

Example 84



15

(i) $\text{Boc-(R)Cgl-Pic-Nag(Z)}$

Prepared from Boc-(R)Cgl-Pic-OH in the same way as described for $\text{Boc-(R)Cha-Pic-Nag(Z)}$ according to method (ic) in Example 20 65.

15 $^1\text{H-NMR}$ (300 MHz, CDCl_3): δ 0.9-1.8 (m, 27H), 2.4 (d, 1H), 3.1-3.3 (m, 5H), 3.9 (d, 1H), 4.2 (t, 1H), 5.1 (s, 2H), 5.2 (bd, 2H), 6.7-7.4 (m, 9H).

25

(ii) $\text{H-(R)Cgl-Pic-Nag(Z)}$

Gaseous hydrogen chloride was bubbled through a solution of $\text{Boc-(R)Cgl-Pic-Nag(Z)}$ (1.38 g, 2.22 mmol) in ethyl acetate 30 (25 ml). After 10 minutes the solvent was evaporated and the residue was dissolved in ethyl acetate and 10% Na_2CO_3 . The organic phase was separated, washed with brine and dried (MgSO_4). Evaporation of the solvent gave 1.02 g (92%) of the title compound.

35

$^1\text{H-NMR}$ (300 MHz, MeOD): δ 1.0-1.9 (m, 18H), 2.2-2.3 (m, 1H), 3.2-3.3 (m, 5H), 3.6 (d, 1H), 3.8-3.9 (bd, 1H), 4.2 (t, 1H),

4.7-4.8 (bs, 5H), 5.1 (s, 2H), 5.2 (s, 1H), 7.2-7.3 (m, 5H).

(iii) $\text{BnOOC-CH}_2\text{-}(R)\text{Cgl-Pic-Nag(Z)}$

5 A solution of the triflate ester of benzyl glycolate (291 mg, 0.98 mmol) in CH_2Cl_2 (2 ml) was added at -25°C to a stirred mixture of $\text{H-}(R)\text{Cgl-Pic-Nag(Z)}$ (0.52 g, 1.04 mmol) and K_2CO_3 (494 mg, 3.58 mmol) in acetonitrile (5 ml) and CH_2Cl_2 (1 ml). The temperature was allowed to reach room temperature during
10 a couple of hours and after 5 days the reaction mixture was diluted with water and extracted with EtOAc and toluene. Drying of the organic phase (MgSO_4) and concentration of the solution gave 319 mg (47%) of colorless crystals.

15 $^1\text{H-NMR}$ (500 MHz, CDCl_3): δ 1.0-1.1 (m, 1H), 1.1-1.3 (m, 4H), 1.35-1.6 (m, 5H), 1.6-1.85 (m, 8H), 1.8-2.2 (bs, 1H), 2.23-2.5 (m, 2H), 2.9 (t, 1H), 3.1-3.5 (m, 6H), 3.6-3.7 (m, 2H), 5.0-5.1 (m, 4H), 5.2 (s, 1H), 6.5-7.4 (m, 13H).

20 (iv) $\text{HOOC-CH}_2\text{-}(R)\text{Cgl-Pic-Nag} \times 2 \text{ HCl}$

$\text{BnOOC-CH}_2\text{-}(R)\text{Cgl-Pic-Nag(Z)}$ (319 mg, 0.49 mmol) was dissolved by heating in isopropanol (50 ml) and water (5 ml) and hydrogenated for 24 h over 10% Pd/C (228 mg). After
25 filtration and evaporation of the solvent and subsequent dissolution in dilute hydrochloric acid followed by freeze drying, the peptide (223 mg, 91%) was isolated as a white powder.

30 $^1\text{H-NMR}$ (500 MHz, D_2O): δ 1.1-2.1 (m, 18H) 2.3 (d, 1H), 3.3 (t, 2H), 3.4 (t, 3H), 3.85-4.05 (m, 3H), 4.6 (d, 1H), 5.15 (s, 1H).

35 $^{13}\text{C-NMR}$ (75 MHz, D_2O): guanidine: δ 157.43 carbonyl carbons:
 δ 169.2, 172.94.

Example 85

H-(R)Hoc-Pro-Nag x 2 TFA

(i) Boc-(R)Hoc-Pro-Nag(Z)

5 Prepared from Boc-(R)Hoc-Pro-OH in the same way as described for Boc-(R)Cha-Pic-Nag(Z) according to Example 65 (ic).

(ii) H-(R)Hoc-Pro-Nag(Z)

10 Prepared in the same way as described for H-(R)Cha-Pro-Agm(Z) (See Example 3).

(iii) H-(R)Hoc-Pro-Nag x TFA

15 Prepared by using the deprotection procedure (a) on the product (ii) above.

$^1\text{H-NMR}$ (300 MHz, D_2O): δ 0.90-1.05 (m, 2H), 1.16-1.48 (m, 6H), 1.48-1.84 (m, 6H), 1.84-2.24 (m, 6H), 2.40 (m, 1H),
20 3.25-3.45 (m, 4H), 3.74 (m, 1H), 3.85 (m, 1H), 4.42 (m, 1H), 4.51 (m, 1H).

Example 86**25 HOOC-CH₂-(R)Hoc-Pro-Nag x HOAc**(i) BnOOC-CH₂-(R)Hoc-Pro-Nag(Z)

Prepared from H-(R)Hoc-Pro-Nag(Z) (See Example 85) according
30 to the procedure described in Example 4.

(ii) HOOC-CH₂-(R)Hoc-Pro-Nag x HOAc

Prepared by using the deprotection procedure (a) on the
35 product (i) above.

$^1\text{H-NMR}$ (300 MHz, D_2O): δ 0.76-0.97 (m, 2H), 1.00-1.37 (m,

6H), 1.50-2.12 (m, 12H) 1.89 (s, acetate), 2.27 (m, 1H), 3.10-3.33 (m, 4H), 3.41 (bs, 2H), 3.61 (m, 1H), 3.77 (m, 1H), 4.12 (m, 1H), 4.37 (m, 1H).

5 ^{13}C -NMR (75 MHz, D_2O): guanidine: δ 157.4; carbonyl carbons: δ 170.8, 173.9, 174.5.

Example 87

10 $\text{HOOC-CH}_2\text{-(R)Hoc-Pic-Nag x HOAc}$

(i) $\text{Boc-(R)Hoc-Pic-Nag(Z)}$

Prepared from Boc-(R)Hoc-Pic-OH in the same way as described
15 for $\text{Boc-(R)Cha-Pic-Nag(Z)}$ according to method (ic) in Example
65.

(ii) $\text{H-(R)Hoc-Pic-Nag(Z)}$

20 Prepared in the same way as described for $\text{H-(R)Cha-Pro-Agm(Z)}$
(See Example 3).

(iii) $\text{BnOOC-CH}_2\text{-(R)Hoc-Pic-Nag(Z)}$

25 Prepared according to the procedure described in Example 4.

(iv) $\text{HOOC-CH}_2\text{-(R)Hoc-Pic-Nag x HOAc}$

Prepared by using the deprotection procedure (a) on the
30 product (iii) above.

$^1\text{H-NMR}$ (300 MHz, D_2O): δ 0.75-0.95 (m, 2H), 1.00-1.30 (m,
6H), 1.30-1.50 (m, 2H), 1.50-1.82 (m, 12H), 1.82-1.95 (bs,
acetate), 2.23 (bd, 1H), 3.08-3.32 (m, 6H), 3.52 (bs, 2H),
35 3.77 (bd, 1H), 4.50 (bs, 1H), 5.00 (bs, 1H).

Example 88

HOOC-CH₂-(R)Dph-Pic-Nag x 2 HCl

(i) Boc-(R)Dph-Pic-Nag(Z)

5 Prepared from Boc-(R)Dph-Pic-OH in the same way as described for Boc-(R)Cha-Pic-Nag(Z) (See Example 65 (ic)).

(ii) H-(R)Dph-Pic-Nag(Z)

10 Prepared in the same way as described for H-(R)Cgl-Pic-Nag(Z) (See Example 84 (ii)).

(iii) BnOOC-CH₂-(R)Dph-Pic-Nag(Z)

15 Prepared from H-(R)Dph-Pic-Nag(Z) according to the procedure described in Example 4.

(iv) HOOC-CH₂-(R)Dph-Pic-Nag x 2 HCl

20 Prepared by using the deprotection procedure (d) on the product (iii) above.

1H-NMR (500 MHz, D₂O): δ 0.46 (m, 1H), 1.2-1.35 (m, 2H), 1.45 (m, 1H), 1.53 (m, 1H), 1.89 (pentet, 2H), 2.03 (bd, 1H), 3.24 (bt, 1H), 3.29 (t, 2H), 3.38 (t, 2H), 3.72 (d, 1H), 3.78 (d, 1H), 3.79 (m, 1H), 4.68 (d, 1H), 4.89 (m, 1H), 5.73 (d, 1H), 7.4-7.6 (m, 6H), 7.65 (t, 2H), 7.81 (d, 2H).

Example 89

30

HOOC-CH₂-(R)Dch-Pic-Nag x HOAc

(i) Boc-(R)Dch-Pic-Nag(Z)

35 Prepared from Boc-(R)Dch-Pic-OH in the same way as described for Boc-(R)Cha-Pic-Nag(Z) (in Example 65 (ic)).

100

(ii) H-(R)Dch-Pic-Nag(Z)

Prepared in the same way as described for H-(R)Cgl-Pic-Nag(Z);
(in Example 84 (ii)).

5

(iii) BnOOC-CH₂-(R)Dch-Pic-Nag(Z)

Prepared from H-(R)Dch-Pic-Nag(Z) according to the procedure
described in Example 4.

10

(iv) HOOC-CH₂-(R)Dch-Pic-Nag x HOAc

Prepared by using the deprotection procedure (a) on the
product (iii) above.

15

¹H-NMR (500 MHz, D₂O): δ 1.2-2.0 (m, 30H), 2.09 (s, acetate),
2.30 (bd, 1H), 3.32 (t, 2H), 3.4-3.5 (m, 3H), 3.65 (d, 1H),
3.70 (d, 1H), 3.86 (bd, 1H), 4.86 (m, 1H), 5.09 (m, 1H).

20

¹³C-NMR (125 MHz, D₂O): guanidine: δ 159.4, carbonyl carbons:
δ 172.5, 173.3, 174.9.

Example P1

25 Solution for parenteral administration

A solution is prepared from the following ingredients:

| | |
|---|-----|
| HOOC-CH ₂ -(R)Cha-Pic-Nag x 2HBr | 5 g |
| Sodium chloride for injection | 9 g |
| Acetic acid | 3 g |
| Water for inj. up to 1000 ml | |

30 The active constituent, the sodium chloride and the acetic
acid are dissolved in the water. The pH is adjusted with 2 M
NaOH to pH 3-7. The solution is filtered through a sterile
0.2 μm filter and is aseptically filled into sterile

ampoules.

Example P2

5 Tablets for oral administration

1000 tablets are prepared from the following ingredients:

| | | |
|----|----------------------------|-------|
| | Thrombin inhibitor | 100 g |
| 10 | Lactose | 200 g |
| | Polyvinyl pyrrolidone | 30 g |
| | Microcrystalline cellulose | 30 g |
| | Magnesium stearate | 6 g |

15 The active constituent and lactose are mixed with an aqueous solution of polyvinyl pyrrolidone. The mixture is dried and milled to form granules. The microcrystalline cellulose and then the magnesium stearate are then admixed. The mixture is then compressed in a tablet machine giving 1000 tablets, each
20 containing 100 mg of active constituent.

Biology

Determination of thrombin clotting time and IC₅₀TT:

25 Human thrombin (T 6769, Sigma Chem Co) in buffer solution, pH 7.4, 100 µl, and inhibitor solution, 100 µl, were incubated for one min. Pooled normal citrated human plasma, 100 µl, was then added and the clotting time measured in an automatic
30 device (KC 10, Amelung).

The clotting time in seconds was plotted against the inhibitor concentration, and the IC₅₀TT was determined by interpolation.

35 IC₅₀TT is the concentration of inhibitor that doubles the thrombin clotting time for human plasma. pIC₅₀TT is the

-log 10 of IC₅₀TT in mol/l. The preferred compounds of the invention have an pIC₅₀TT in the range 6.6 - 8.2.

Determination of Activated Partial Thromboplastin Time (APTT)

5

APTT was determined in pooled normal human citrated plasma with the reagent PTT Automated 5 manufactured by Stago. The inhibitors were added to the plasma (10 µl inhibitor solution to 90 µl plasma) and APTT was determined in the mixture by 10 use of the coagulation analyser KC10 (Amelung) according to the instructions of the reagent producer. The clotting time in seconds was plotted against the inhibitor concentration in plasma and the IC₅₀APTT was determined by interpolation.

15 IC₅₀APTT is defined as the concentration of inhibitor in plasma that doubled the Activated Partial Thromboplastin Time. pIC₅₀APTT is the -log 10 of IC₅₀APTT in mol/l. Those of the preferred compounds of the invention that were tested showed a pIC₅₀APTT of 5.1 - 6.4.

20

ABBREVIATIONS

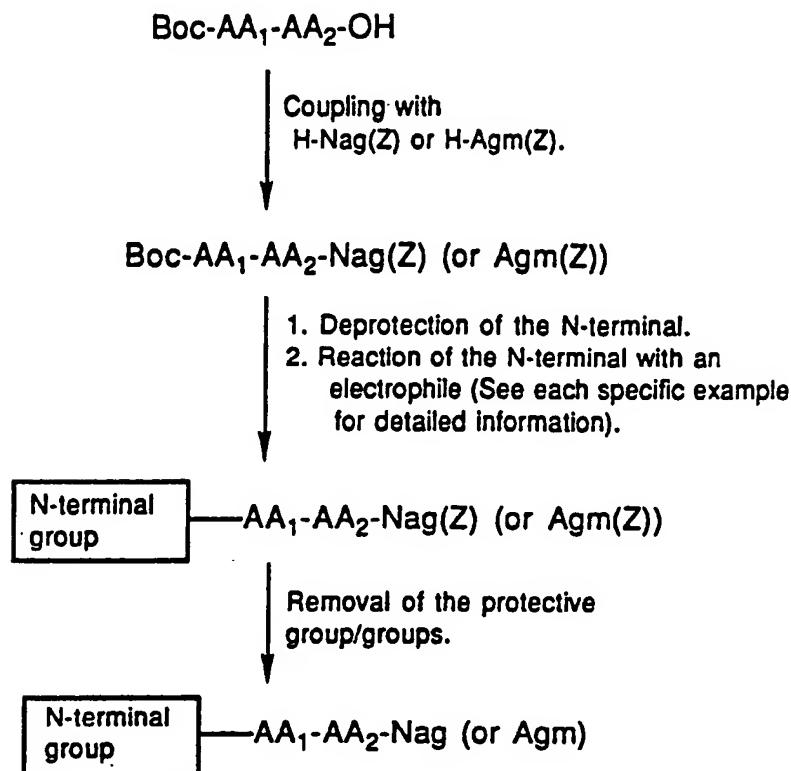
| | |
|----------------------|--|
| Agm = | Agmatine |
| Agm(Z) = | ω-N-benzyloxycarbonyl agmatine |
| 25 AA ₁ = | Amino acid 1 |
| AA ₂ = | Amino acid 2 |
| Aze = | (S)-Azetidin-2-carboxylic acid |
| Bla = | α-substituted butyrolactone |
| Boc = | tertiary butoxy carbonyl |
| 30 Brine = | saturated water/NaCl solution |
| Bu = | butyl |
| Bn = | benzyl |
| Cgl = | (S)-Cyclohexyl glycine |
| Ch = | cyclohexyl |
| 35 Cha = | (S)-β-cyclohexyl alanine |
| CME-CDI = | 1-Cyclohexyl-3-(2-morpholinoethyl) carbodiimide metho-p-toluenesulfonate |

| | | |
|----|----------|---|
| | DCC = | dicyclohexyl carbodiimide |
| | Dch = | (S)-Dicyclohexyl alanine |
| | DMAP= | N,N-dimethyl amino pyridine |
| | DMF = | dimethyl formamide |
| 5 | DMSO = | dimethyl sulphoxide |
| | Dph = | (S)-Diphenyl alanine |
| | EDC = | 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride |
| | Et = | ethyl |
| 10 | EtOAc = | ethyl acetate |
| | HOAc = | acetic acid |
| | HOBt = | N-hydroxy benzotriazole |
| | Hoc = | (S)-Homocyclohexyl alanine |
| | Hop = | (S)-Homophenyl alanine |
| 15 | HOSu = | N-hydroxysuccinimide |
| | Mag = | miniagmatine |
| | Me = | methyl |
| | Mor = | (S)-morpholine-2-carboxylic acid |
| | Mpa = | mega pascal |
| 20 | Nag = | noragmatine |
| | Nag(Z) = | δ -N-benzyloxycarbonyl-noragmatine |
| | NMM = | N-methyl morpholine |
| | Pgl = | (S)-phenyl glycine |
| | Ph = | phenyl |
| 25 | Phe = | (S)-phenyl alanine |
| | Pic = | (S)-pipecolinic acid |
| | Pr = | propyl |
| | Pro = | (S)-proline |
| | RPLC = | reverse phase high- performance liquid chromatography |
| 30 | Tf = | trifluoromethyl sulphonyl |
| | TFA = | trifluoracetic acid |
| | THF = | tetrahydrofuran |
| | p-TsOH = | para-toluenesulfonic acid |
| 35 | Val = | (S)-valine |
| | Z = | benzyloxy carbonyl |

104

Prefixes n, s, i and t have their usual meanings: normal,
iso, sec and tertiary.

**Scheme I (Example 3-18,20-21,24-28,30-34,36-40,43-49,
51-53,57-64 and 67-93)**



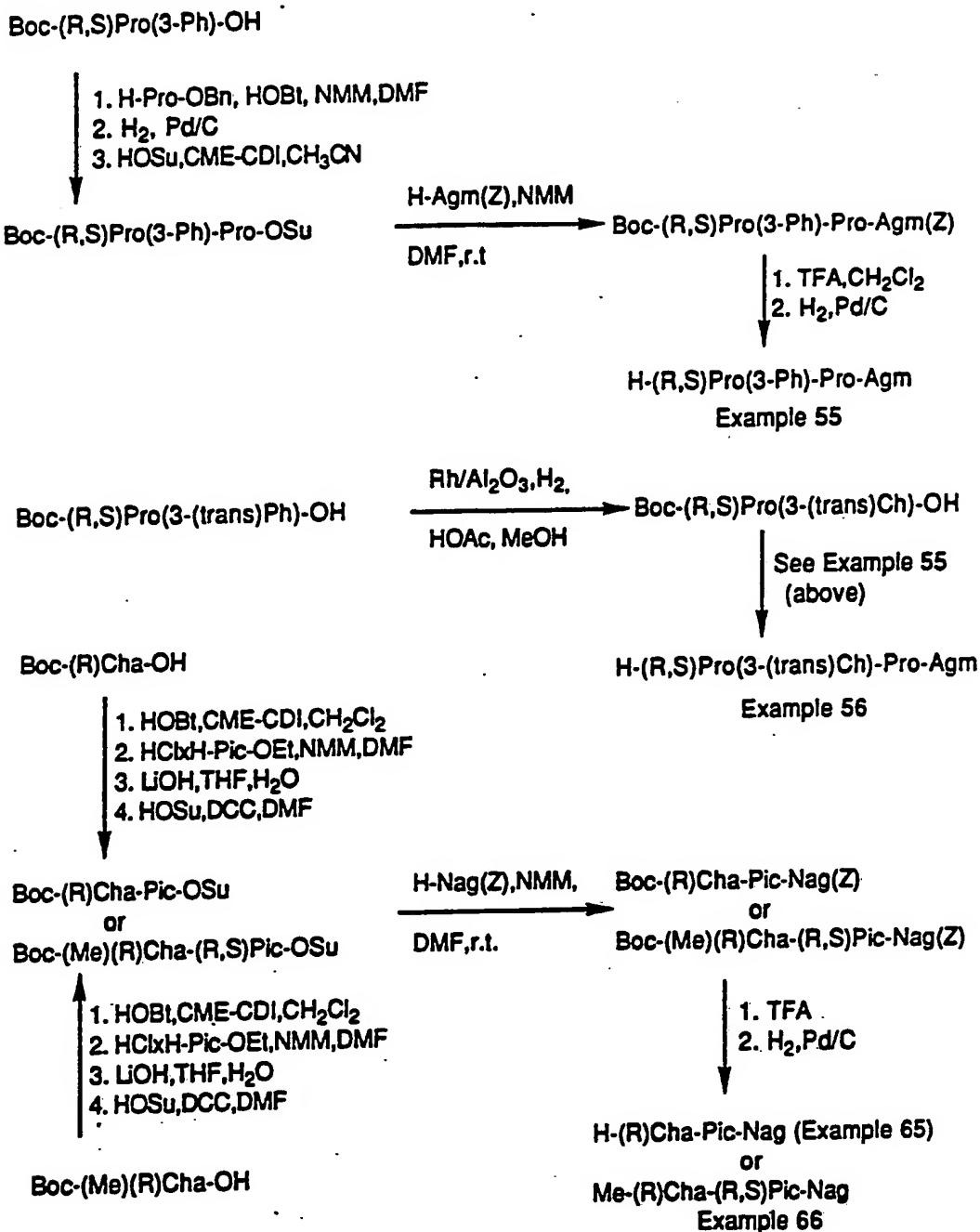
AA₁= H-(R)Cha-OH, Me-(R)Cha-OH, H-(R,S)Pro(3-(trans)Ph)-OH,
H-(R)Hoc-OH, H-(R)Cgl-OH, H-(R)Dph-OH, H-(R)Dch-OH

AA₂= H-Pro-OH, H-Pic-OH, H-Mor-OH, H-Aze-OH, H-Pic(4-(S)Me)-OH
H-Pic(4-(R)Me)-OH, H-(R,S)Pic(4,5-dehydro)-OH,
H-(R)Pic(4-(R)Me)-OH, H-Pro(5-(R,S)Me)-OH,
H-Pro(5-(S)Me)-OH, H-Pic(6-(S)Me)-OH

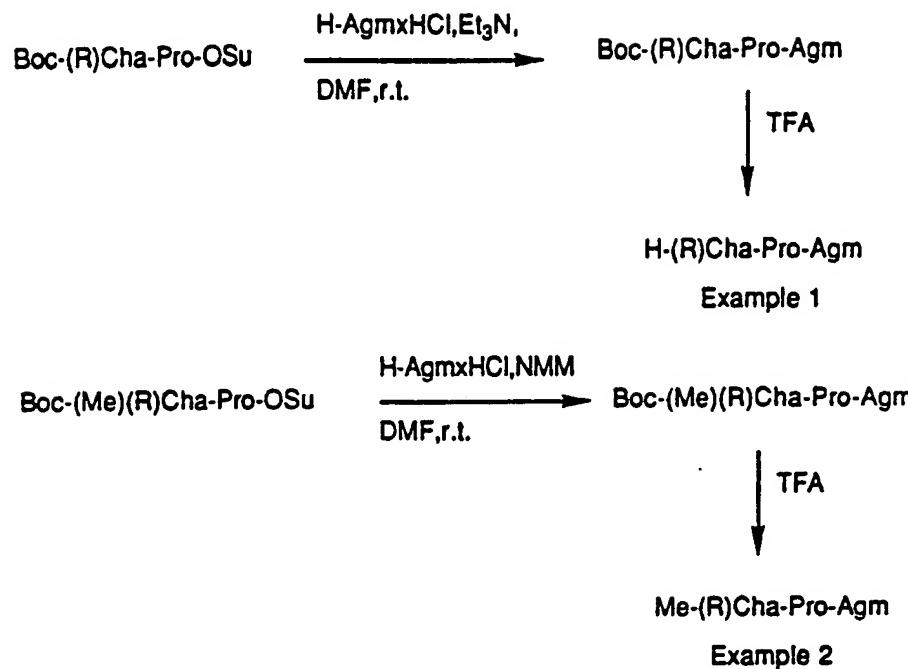
The N terminal group in the final compound =

H, HO-(CH₂)₃-, ^tBu-, HOOC-CH₂, MeOOC-CH₂-, ^tPrOOCH₂-, ^tBuOOCH₂-,
HOOC-CH(Me)-, HOOC-CH(^tPr)-, HOOC-CH(Ph)-, HOOC-CH(CH₂CH₂Ph)-
HOOC-CH₂CH₂-, HOOC-CH₂CH₂CH₂-, EtOOCH₂CH₂CH₂-, Bla,
HOOC-CH₂OOC-CH₂-, EtOOOC-CO, MeOOC-CO, HOOC-CO-, H₂NOC-CH₂-
HOOC-CH(CH₂COOH)-, MeOOC-CH(CH₂COOMe), HOOC-CH₂NH-CO-CH₂-,
HOOC-CH(CH₂OH)-, (HO)₂P(O)-CH₂-, EtO(HO)P(O)-CH₂-,
(EtO)₂P(O)-CH₂-,

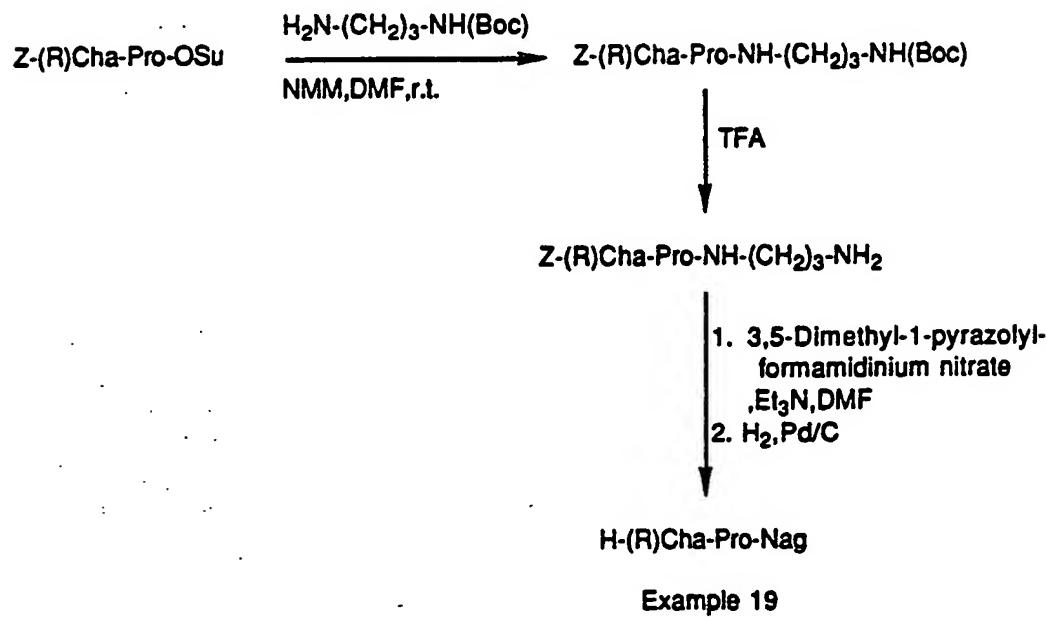
Scheme II (Example 55,56,65 and 66)

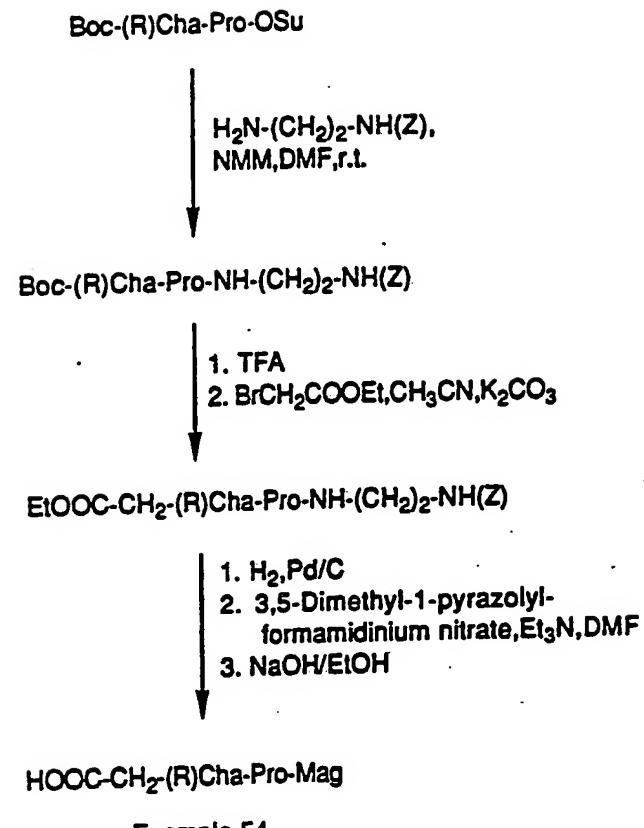


Scheme III (Example 1 and 2)

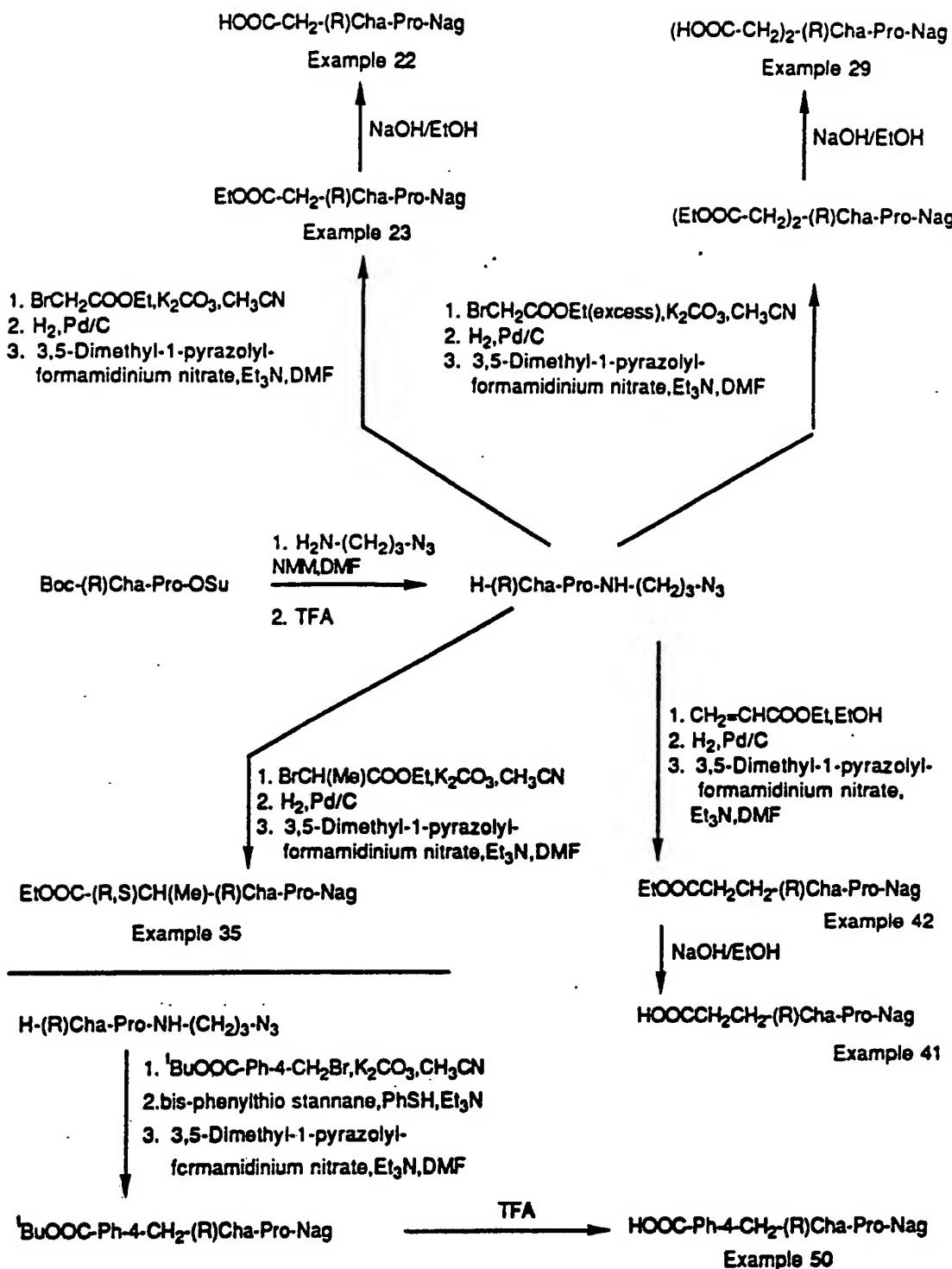


Scheme IV (Example 19)



Scheme V (Example 54)**Example 54**

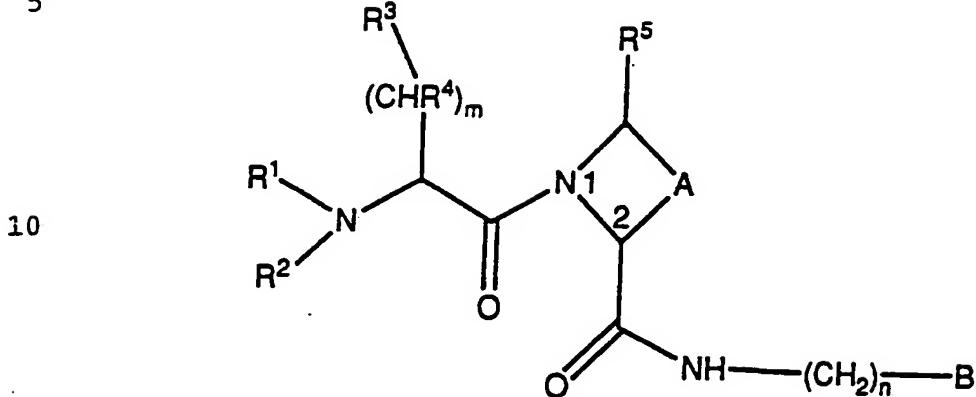
Scheme VI (Example 22,23,29,35,41,42 and 50)



CLAIMS

1. A compound of the general formula

5



10

Formula I

20 wherein:

A represents a methylene group, or

25 A represents an ethylene group and the resulting 5-membered ring may or may not carry one or two fluorine atoms, a hydroxy group or an oxo group in position 4, or may or may not be unsaturated, or

30 A represents -CH₂-O-, -CH₂-S-, -CH₂-SO-, with the heteroatom functionality in position 4, or

35 A represents a n-propylene group and the resulting 6-membered ring may or may not carry in position 5 one fluorine atom, a hydroxy group or an oxo group, carry two fluorine atoms in one of positions 4 or 5 or be unsaturated in position 4 and 5, or carry in position 4 an alkyl group with 1 to 4 carbon atoms, or

A represents $\text{-CH}_2\text{-O-CH}_2\text{-}$, $\text{-CH}_2\text{-S-CH}_2\text{-}$, $\text{-CH}_2\text{-SO-CH}_2\text{-}$;

R¹ represents H, an alkyl group having 1 to 4 carbon atoms, a hydroxyalkyl group having 2-3 carbon atoms or R¹¹OOC-alkyl-,
5 where the alkyl group has 1 to 4 carbon atoms and R¹¹ is H or an alkyl group having 1 to 4 carbon atoms or an alkylene group having 2-3 carbon atoms intramolecularly bound alpha to the carbonyl group in R¹, or

10 R¹ represents R¹²OOC-1,4-phenyl-CH₂-, where R¹² is H or an alkyl group having 1 to 4 carbon atoms, or

15 R¹ represents R¹³-NH-CO-alkyl-, where the alkyl group has 1 to 4 carbon atoms and is possibly substituted alpha to the carbonyl with an alkyl group having 1 to 4 carbon atoms and where R¹³ is H or an alkyl group having 1 to 4 carbon atoms or -CH₂COOR¹², where R¹² is as defined above, or

20 R¹ represents R¹²OOC-CH₂-OOC-alkyl-, where the alkyl group has 1 to 4 carbon atoms and is possibly substituted alpha to the carbonyl with an alkyl group having 1 to 4 carbon atoms and where R¹² is as defined above, or

25 R¹ represents CH₃SO₂-, or

30 R¹ represents R¹²OCOCO- where R¹² is as defined above, or

R¹ represents -CH₂PO(OR¹⁴)₂, -CH₂SO₃H or -CH₂-(5-(1H)-tetrazolyl) where R¹⁴ is, individually at each occurrence, H, methyl or ethyl;

35 R² represents H or an alkyl group having 1 to 4 carbon atoms or R²¹OOC-alkyl-, where the alkyl group has 1 to 4 carbon atoms and is possibly substituted in the position which is alpha to the carbonyl group, and the alpha substituent is a group R²²-(CH₂)_p-⁻, wherein p = 0-2 and R²² is methyl, phenyl, OH, COOR²¹, and R²¹ is H or an alkyl group having 1 to 4

carbon atoms;

m is 0, 1 or 2, R³ represents a cyclohexyl group and R⁴ represents H, or

5

m is 1 and R³ represents a cyclohexyl or phenyl group and R² forms an ethylene bridge together with R¹, or

10 m is 1 and R³ and R⁴ each represents a cyclohexyl or phenyl group;

R⁵ represents H or an alkyl group having 1 to 4 carbon atoms;

n is an integer 2 to 6; and

15

B represents -N(R⁶)-C(NH)-NH₂, wherein R⁶ is H or a methyl group, or

B represents -S-C(NH)-NH₂, or -C(NH)-NH₂,

20

either the compound as such or in the form of a physiologically acceptable salt and including stereoisomers.

25

2. A compound according to claim 1 wherein R¹ represents R¹¹OOC-alkyl-, where the alkyl group has 1 to 4 carbon atoms and R¹¹ is H.

3. A compound according to claim 2 wherein A is ethylene and R⁵ is H or an alkyl group having 1 to 4 carbon atoms.

30

4. A compound according to claim 2 wherein A is n-propylene and the resulting 6-membered ring may or may not carry in position 4 on alkyl group with 1 to 4 carbon atoms, and R⁵ is H or an alkyl group having 1 to 4 carbon atoms.

35

5. A compound according to one or more of the preceding claims wherein R³ is cyclohexyl, m is 1, 2 and R⁴ is H.

6. A compound according to one or more of the preceding claims wherein n is 3.

7. A compound according to one or more of the proceeding 5 claims having S-konfiguration on the α -amino acid in the P2 position.

8. A compound according to claim 7 having R-konfiguration on the α -amino acid in the P3 position.

10 9. A compound selected from

H- (R) Cha-Pro-Agm
Me- (R) Cha-Pro-Agm
15 HO- (CH₂)₃ - (R) Cha-Pro-Agm
ⁱPrOOC-CH₂ - (R) Cha-Pro-Agm
HOOC- (R, S) CH(Me) - (R) Cha-Pro-Agm
HOOC- (R or S) CH(Me) - (R) Cha-Pro-Agm/a
HOOC- (R or S) CH(ⁿPr) - (R) Cha-Pro-Agm/a
20 HOOC- (R or S) CH(ⁿPr) - (R) Cha-Pro-Agm/b
HOOC- (R or S) CH(Ph) - (R) Cha-Pro-Agm/b
HOOC- (R, S) CH(CH₂CH₂Ph) - (R) Cha-Pro-Agm
HOOC- (R or S) CH(CH₂CH₂Ph) - (R) Cha-Pro-Agm/a
HOOC-CH₂-CH₂ - (R) Cha-Pro-Agm
25 EtoOC-CO- (R) Cha-Pro-Agm
(R, S) Bla- (R) Cha-Pro-Agm
HOOC- (R or S) CH(CH₂CH₂Ph) - (R) Cha-Pro-Agm/b
H- (R) Cha-Pro-Nag
ⁿBu- (R) Cha-Pro-Nag
30 HO- (CH₂)₃ - (R) Cha-Pro-Nag
EtoOC-CH₂ - (R) Cha-Pro-Nag
ⁱPrOOC-CH₂ - (R) Cha-Pro-Nag
^tBuOOC-CH₂ - (R) Cha-Pro-Nag
HOOC-CH₂-OOC-CH₂ - (R) Cha-Pro-Nag
35 H₂N-CO-CH₂ - (R) Cha-Pro-Nag
HOOC-CH₂-NH-CO-CH₂ - (R) Cha-Pro-Nag
(HOOC-CH₂)₂ - (R) Cha-Pro-Nag

HOOC-CH₂- (nBu) (R) Cha-Pro-Nag
HOOC- (R, S) CH(Me) - (R) Cha-Pro-Nag
HOOC- (R or S) CH(Me) - (R) Cha-Pro-Nag/a
EtOOC- (R, S) CH(Me) - (R) Cha-Pro-Nag
5 HOOC- (R or S) CH(ⁿPr) - (R) Cha-Pro-Nag/a
HOOC- (R) CH(CH₂-OH) - (R) Cha-Pro-Nag
HOOC- (R, S) CH(Ph) - (R) Cha-Pro-Nag
HOOC- (S) CH(CH₂CH₂Ph) - (R) Cha-Pro-Nag
HOOC- (R) CH(CH₂CH₂Ph) - (R) Cha-Pro-Nag
10 HOOC-CH₂-CH₂- (R) Cha-Pro-Nag
EtOOC-CH₂-CH₂- (R) Cha-Pro-Nag
HOOC- (CH₂)₃- (R) Cha-Pro-Nag
EtOOC- (CH₂)₃- (R) Cha-Pro-Nag
HOOC-CO- (R) Cha-Pro-Nag
15 MeOOC-CO- (R) Cha-Pro-Nag
(R, S) Bla- (R) Cha-Pro-Nag
HOOC- (R, S) CH(CH₂COOH) - (R) Cha-Pro-Nag
MeOOC- (R, S) CH(CH₂COOME) - (R) Cha-Pro-Nag
HOOC-Ph-4-CH₂- (R) Cha-Pro-Nag
20 (HO)₂P(O)-CH₂- (R) Cha-Pro-Nag
EtO(HO)P(O)-CH₂- (R) Cha-Pro-Nag
(EtO)₂P(O)-CH₂- (R) Cha-Pro-Nag
HOOC-CH₂- (R) Cha-Pro-Mag
H- (R, S) Pro(3-Ph) -Pro-Agm
25 H- (R, S) Pro(3- (trans) Ch) -Pro-Agm
HOOC-CH₂- (R, S) Pro(3- (trans) Ph) -Pro-Agm
HOOC-CH₂- (R, S) Pro(3- (trans) Ph) -Pro-Nag
HOOC-CH₂- (Me) (R) Cha- (R, S) Pic-Agm
HOOC- (R, S) CH(Me) - (R) Cha-Pic-Agm
30 HOOC- (R or S) CH(Me) - (R) Cha-Pic-Agm/a
HOOC-CH₂-CH₂- (R) Cha-Pic-Agm
H- (R) Cha-Pic-Nag
Me- (R) Cha- (R, S) Pic-Nag
MeOOC-CH₂- (R) Cha-Pic-Nag
35 iPrOOC-CH₂- (R) Cha-Pic-Nag
HOOC-CH₂- (Me) (R) Cha- (R or S) Pic-Nag/b
HOOC- (R, S) CH(Me) - (R) Cha- (R, S) Pic-Nag

HOOC-(RorS)CH(Me)-(R)Cha-(RorS)Pic-Nag/c
HOOC-CH₂-CH₂-(R)Cha-Pic-Nag
HOOC-CH₂-(R)Cha-(R,S)Mor-Agm
HOOC-CH₂-(R)Cha-(RorS)Mor-Nag
5 H-(R)Cha-Aze-Nag
HOOC-CH₂-(R)Cha-Aze-Nag
H-(R)Cha-Pro(5-(S)Me)-Nag
HOOC-CH₂-(R)Cha-(RorS)Pic(4,5-dehydro)-Nag/b
HOOC-CH₂-(R)Cha-(R)Pic(4-(R)Me)-Nag
10 HOOC-CH₂-(R)Cgl-Pic-Nag
H-(R)Hoc-Pro-Nag
HOOC-CH₂-(R)Hoc-Pro-Nag
HOOC-CH₂-(R)Hoc-Pic-Nag
HOOC-CH₂-(R)Dph-Pic-Nag
15 HOOC-CH₂-(R)Dch-Pic-Nag
HOOC-CH₂-(R)Cha-Pro(5-(R,S)Me)-Nag
HOOC-CH₂-(R)Cha-Pic(4-(R)Me)-Nag
H-(R)Cha-Pic(4-(R)Me)-Nag
HOOC-CH₂-(R)Cha-Pic(6-(S)Me)-Nag
20 either as such or in the form of a physiologically acceptable salt and including stereoisomers.

10. A compound selected from
HOOC-CH₂-(R)Cha-Pro-Agm
HOOC-CH₂-(Me)(R)Cha-Pro-Agm
5 HOOC-(RorS)CH(Me)-(R)Cha-Pro-Agm/b
HOOC-CH₂-(R)Cha-Pro-Nag
HOOC-CH₂-(R)Cha-Pic-Agm
HOOC-(RorS)CH(Me)-(R)Cha-Pic-Agm/b
HOOC-(RorS)CH(Me)-(R)Cha-(RorS)Pic-Nag/d
10 HOOC-CH₂-(R)Cha-Pro(5-(S)Me)-Nag
HOOC-CH₂-(R)Cha-Pic(4-(S)Me)-Nag

either as such or in the form of a physiologically acceptable salt and including stereoisomers.

15

11. The compound

HOOC-CH₂-(Me)(R)Cha-Pro-Nag,

either as such or in the form of a physiologically acceptable salt and including stereoisomers.

20

12. The compound

HOOC-(RorS)CH(Me)-(R)Cha-Pro-Nag/b,

either as such or in the form of a physiologically acceptable salt and including stereoisomers.

25

13. The compound

HOOC-CH₂-(R)Cha-Pic-Nag

either as such or in the form of a physiologically acceptable salt and including stereoisomers.

30

14. A process for preparing a compound according to any of claims 1-13, which process comprises coupling of an N-terminally protected amino acid or dipeptide or a preformed, N-terminally alkylated protected dipeptide to a compound

35

H₂N-(CH₂)_n-X

wherein n is an interger 2 to 6 and X is an unprotected or protected guanidino group or a protected amino group, or a group transferable into an amino group, where the amino group is subsequently transferred into a guanidino group,

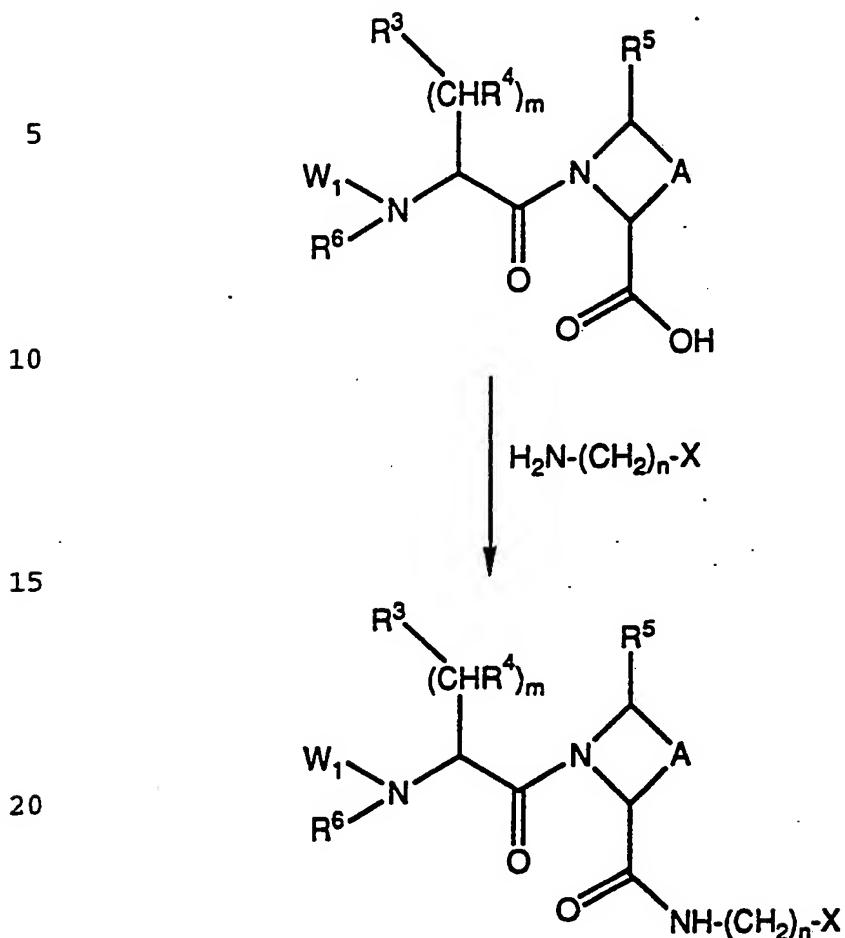
5

and if desired forming a physiologically acceptable salt, and in those cases where the reaction results in a mixture of stereoisomers, these are optionally separated by standard chromatographic or re-crystallisation techniques, and if
10 desired a single stereoisomer is isolated.

15. A process according to claim 14 for preparing a compound according to any of claims 1-13, which process comprises:

15 a) (Method I) Coupling of an N-terminally protected dipeptide with either a protected- or unprotected amino guanidine or a straight chain alkylamine carrying a protected or masked amino group at the terminal end of the alkyl chain, using

standard peptide coupling, as shown in the formula:



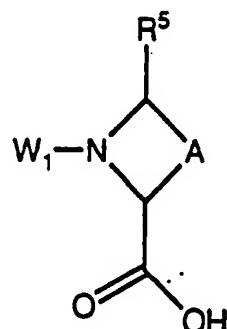
25 wherein R^3 , R^4 , R^5 , n, m and A are as defined in Formula I, R^6 is H or alkyl, W_1 is an amino protecting group such as tertiarybutoxy carbonyl and benzyloxy carbonyl and X is $-NH-C(NH)NH_2$, $-NH-C(NH)NH-W_2$, $-N(W_2)-C(NH)NH-W_2$,

30 -NH-C(NW₂)₂-NHW₂ or -NH-W₂, where W₂ is an amine protecting group such as tertiarybutoxy carbonyl or benzyloxy carbonyl, or X is a masked amino group such as azide, giving the protected peptide, or

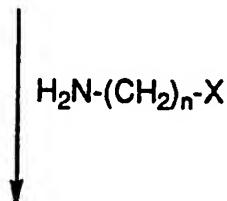
35 b) (Method II) Coupling of an N-terminally protected amino acid. with either a protected- or unprotected amino guanidine

or a straight chain alkylamine carrying a protected or masked amino group at the terminal end of the alkyl chain, using standard peptide coupling, as shown in the formula

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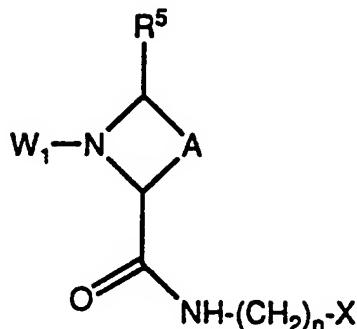


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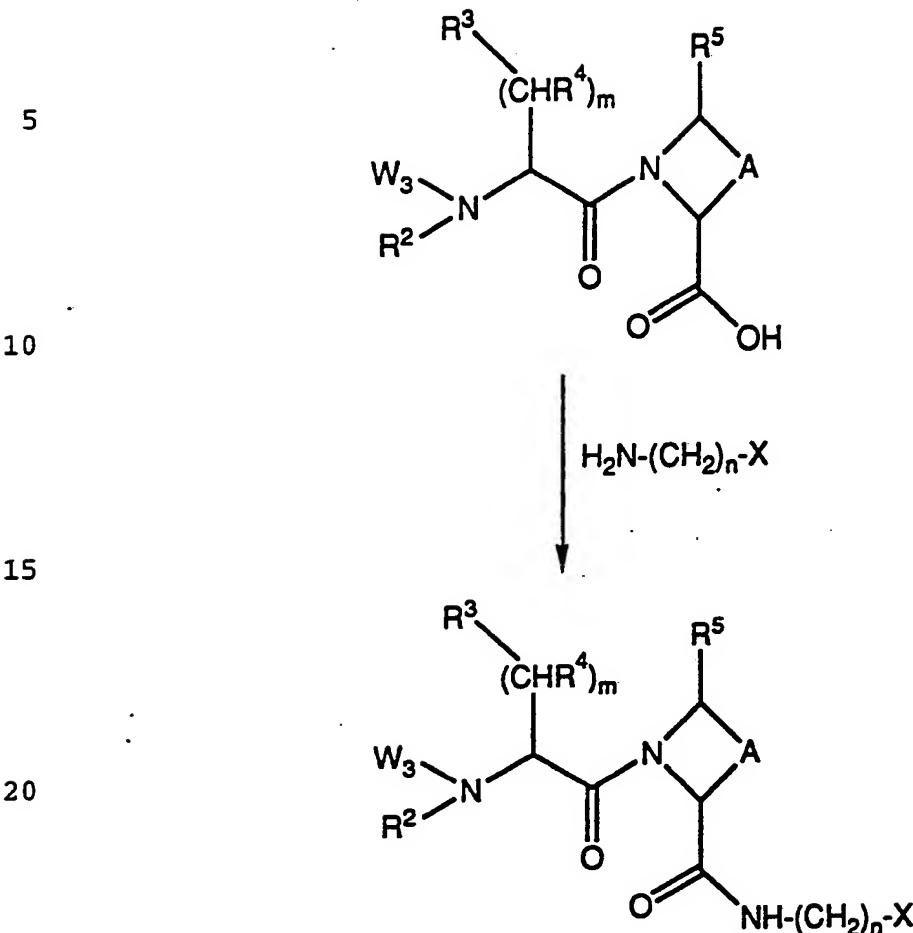
25

wherein W_1 , A, R^5 and X are as defined above followed by deprotection of the W_1 -group and coupling with the N-terminal 30 amino acid, in a protected form, or

c) (Method III) Coupling of a preformed N-terminally alkylated and protected dipeptide, prepared by standard peptide coupling, with either a protected or unprotected 35 amino guanidine or a straight chain alkylamine carrying a protected or masked aminogroup at the terminal end of the alkyl chain, using standard peptide coupling, as shown in the

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formula



wherein R^2 , R^3 , R^4 , R^5 , n , m , A and X are defined as above provided that R^2 is other than H and W_3 is an acyl protecting group such as trifluoroacetyl,

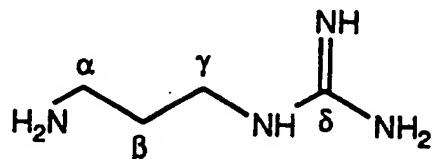
30 whereupon the final compounds are made in any of the following ways, depending on the nature of the X - group used:
Removal of the protecting group(s) (when $X = -NH-C(NH)NH_2$, $NH-C(NH)NH-W_2$, $-N(W_2)-C(NH)NH-W_2$, $-NH-C(NW_2)NH-W_2$) or a selective deprotection of the W_1 - group (e.g when $X =$
35 $-NH-C(NH)NH-W_2$, $-N(W_2)-C(NH)NH-W_2$, $-NH-C(NW_2)NH-W_2$, W_2 in this case must be orthogonal to W_1) followed by alkylation of the N-terminal nitrogen and deprotection or a selective

deprotection/unmasking of the terminal alkylamino function (X= NH-W₂, W₂ in this case must be orthogonal to W₁ and W₃, respectively, or X= a masked aminogroup, such as azide) followed by a guanidation reaction, using standard methods, 5 of the free amine and deprotection of the W₁- or W₃-group, respectively,

and if desired forming a physiologically acceptable salt, and in those cases where the reaction results in a mixture of 10 stereoisomers, these are optionally separated by standard chromatographic or re-crystallisation techniques, and if desired a single stereoisomer is isolated.

16. Use of a compound of the formula:

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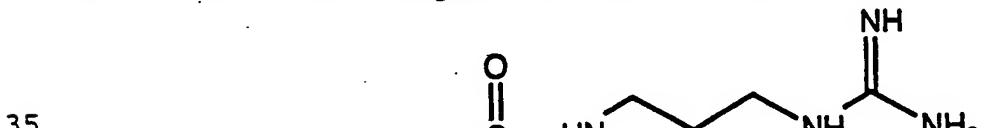


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either as such or in the form of a salt, and as such or having the guanidino group either mono protected at the δ-nitrogen or diprotected at the δ-nitrogens or the γ, δ-nitrogens, as a starting material in synthesis of a serine protease inhibitor, and in particular in synthesis of a thrombin inhibitor.

17. Use according to claim 16, where the serine protease 30 inhibitor is a peptidic compound.

18. A structural fragment of the formula



as a structural element in a pharmaceutically active compound.

19. A structural fragment according to claim 18 where the pharmaceutically active compound is a peptidic compound.
20. A compound according to any of claims 1-13 for use in therapy.
21. A compound according to claim 20 for use as an anticoagulant or antithrombotic agent.
- 10 22. A pharmaceutical preparation comprising an effective amount of a compound as outlined in claims 1-13 in conjunction with one or more pharmaceutical carriers.
- 15 23. A pharmaceutical preparation according to claim 22 for use as an anticoagulant or antithrombotic agent.
- 20 24. Use of compound according to any of claims 1-13 as an active ingredient for manufacture of a pharmaceutical preparation for inhibition of thrombin in a human or animal organism.
- 25 25. A method for obtaining inhibition of thrombin in a human or animal organism in need of such inhibition, comprising administering to said organism an inhibitory effective amount of a compound claimed in any of claims 1-13.
- 30 26. A method of treatment or prophylaxis of thrombosis and hypercoagulability in blood and tissues in a human or animal organism, comprising administering to a host in need of such treatment or prophylaxis an effective amount of a compound claimed in any of claims 1-13.
- 35 27. A compound, a process, a pharmaceutical preparation, a use and a method as claimed in any of claims 1-26 and substantially as described.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/SE 92/00832

A. CLASSIFICATION OF SUBJECT MATTER

IPC5: C07K 5/06, A61K 37/64, C07K 5/04 // C 07 C 279/12
 According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC5: A61K, C07C, C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

BIOSIS, EMBASE, MEDLINE, WPI, CHEMICAL ABSTRACTS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|-----------|---|-----------------------|
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| A | GB, A, 2085444 (RICHTER GEDEON, VEGYESZETI GYAR RT), 28 April 1982 (28.04.82) | 1-15,18-24 |
| Y | -- | 16,17 |
| Y | EP, A1, 0074787 (SMITHKLINE BECKMAN CORPORATION), 23 March 1983 (23.03.83), see example 8 and claim 11 -- | 16,17 |

 Further documents are listed in the continuation of Box C. See patent family annex.

- * Special categories of cited documents:
- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed
- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
- "&" document member of the same patent family

Date of the actual completion of the international search

16 April 1993

Date of mailing of the international search report

19 -04- 1993

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/SE 92/00832

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
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| A | EP, A2, 0235692 (BEHRINGWERKE AKTIENGESELLSCHAFT), 9 Sept 1987 (09.09.87) -- | 1-15,18-24 |
| P,A | WO, A1, 9207869 (THROMBOSIS RESEARCH INSTITUTE), 14 May 1992 (14.05.92) ----- | 1-15,18-24 |

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Information on patent family members

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| International application No. | |
| PCT/SE 92/00832 | |

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
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